1903

Compliments of the Author

Samuel M. Bain.

OF THE

Agricultural Experiment Station

OF THE

UNIVERSITY OF TENNESSEE

VOL. XV APRIL, 1902 No. 2

IMPERIAL BUREAU OF MYCOLOGY 28 JUN. 1921

THE ACTION OF COPPER ON LEAVES

With Special Reference to the Injurious Effects of Fungicides on Peach Foliage

A Physiological Investigation

KNOXVILLE, TENNESSEE

THE AGRICULTURAL EXPERIMENT STATION

OF THE UNIVERSITY OF TENNESSEE

CHARLES W. DABNEY, *President and Director*

EXECUTIVE COMMITTEE

J. W. CALDWELL
O. P. TEMPLE
T. F. P. ALLISON
T. E. HARWOOD
HARRIS BROWN
J. B. KILLEBREW

TREASURER
JAMES MAYNARD

SECRETARY
J. W. GAUT

STATION OFFICERS

CHARLES W. DABNEY, President
ANDREW M. SOULE, Vice-Director and Agriculturist
SAMUEL M. BAIN, Botanist
CHARLES A. KEFFER, Horticulturist
CHARLES A. MOOERS, Chemist
WESTON M. FULTON, Meteorologist
MOSES JACOB, Consulting Veterinarian
JOHN R. FAIN, Farm Manager
PHARES O. VANATTER, Assistant for Plat Work
SAMUEL E. BARNES, Dairyman
FREDERICK H. BROOME, Librarian
MISS ETHEL REESE, Stenographer

The station has facilities for analyzing and testing fertilizers, cattle foods, milk and dairy products; seeds with reference to their purity or germinating power; for identifying grasses and weeds, and studying forage plants; for investigating the diseases of fruits and fruit trees, grains and other useful plants.

Packages by express, to receive attention, should be prepaid.

All communications should be addressed to the

AGRICULTURAL EXPERIMENT STATION,
Knoxville, Tennessee.

☞The experiment station building, containing its offices, laboratories and museum, and the plant house and horticultural department, are located on the University grounds, 15 minutes walk from the Custom House in Knoxville. The experiment farm, dairy barn, stables, milk laboratory, etc., are located one mile west of the University, on the Kingston pike. The fruit farm is adjacent to the Industrial School, easily reached by the Middlebrook car line. Farmers are cordially invited to visit the buildings and experimental grounds.

Bulletins of this station will be sent, upon application, free of charge, to any farmer in the state.

CONTENTS

	Page
Introduction	21
Chapter I, Poisonous Action of Copper Solutions Introduced through the Roots	23
Chapter II, Poisonous Action of Copper on the Leaves and Certain Conditions Affecting this Action	27
Soluble Copper Salts	27
Pure Basic Copper	28
Effect of Various Admixtures with Basic Copper	36
Effect of Rain, Dew, Sunlight, and Hygroscopic Moisture	44
Chapter III, Permeability of the Cuticle and its Relation to the Action of Fungicides	53
Chapter IV, Wound-Healing of the Leaves and Leaf-Fall	69
Chapter V, Influence of Copper on the Assimilative Function of Leaves	74
Chapter VI, General Conclusions and Practical Application of Results	88
Bibliography	101
Description of Plates	107

Digitized by the Internet Archive
in 2025

https://archive.org/details/bulletin-ut-agricultural-experiment-station_1902-04_15_2

THE ACTION OF COPPER ON LEAVES

With Special Reference to the Injurious Effects of Fungicides on Peach Foliage: A Physiological Investigation

SAMUEL M. BAIN, Botanist

INTRODUCTION

The investigations recorded in the succeeding chapters are the outcome of some preliminary experiments made at the Tennessee Experiment Station* in the year 1895 with a view to gaining some knowledge as to the effect of fungicides on peach foliage, which might be of some economic value in the treatment of the disease of the peach commonly known as Brown Rot. Unavoidable interruptions interfered somewhat with the progress of the work, and the results here published were obtained for the most part during the seasons of 1899, 1900, and 1901. A very large proportion of the experimental work led to no fruitful results, and no reference is made to such in the text.

The general lines upon which the work was planned at the outset are readily suggested by the table of contents. The rational method of procedure was, in the first place, to select for a comparative study with the peach plant several others whose foliage is not susceptible to injury by fungicides. For this purpose the grape and apple seemed best fitted for various reasons.

The first problem presented was, Which of these three plants is the more susceptible to copper poison when introduced through a part of the plant not covered by a thick cuticle? Is the peach leaf less protected by such cuticle than the apple and grape leaf? The solution of these problems involves a thorough investigation of the subject from various points of view, leading into several divergent lines of purely physiological investigation.

By a comparison of these three plants from various physiological and histological points of view, it was hoped to gain some information that would lead to some practically beneficial result, or at least to a clearer understanding of the real problem underlying the whole investigation: *Why is the foliage of the peach more susceptible to injury by fungicides than that of other plants?*

Naturally, an investigation leading out into so many different directions demands the study of a wide range of physiological and horticultural literature. The writer by no means makes pretension to having exhausted the literature in all these different fields, but it is believed that enough has usually been cited under the discussion of each subject to establish the

*Bull. Tenn. Exp. Sta., vol. 8, No. 3, 1895, pp. 35–40.

point at issue. No effort whatever has been made to review the literature of any part of the subject, and that cited has been solely for the sake of evidence. The various works consulted in the course of the investigation, which have direct bearing on the question, are found in the bibliography on p. 101.

A preliminary report on this work was published in *Science,* N. S., vol. 14, 1901, pp. 221, 222. A brief discussion of some points may be found in *The Botanical Gazette,* vol. 33, 1902, pp. 244, 245.

The writer wishes to acknowledge his obligations to several of his colleagues, to whom he is indebted for advice at several stages of the experimental part of the work, including Dr Charles A. Perkins, Professor of Physics and Electrical Engineering; Dr Charles E. Wait, Professor of Chemistry; and Mr C. A. Mooers, Chemist of the Experiment Station. Professor W. M. Fulton kindly placed at the author's disposal the official weather record for Knoxville. At the writer's request, Miss E. L. Ogden, Assistant Librarian in the U. S. Department of Agriculture, very kindly consulted the various current periodicals in the Department library just before going to press. The writer wishes finally to express his great indebtedness to Dr A. C. True and other gentlemen of the Office of Experiment Stations, through whom he has had access to the works in the Department library at Washington.

CHAPTER I

Poisonous Action of Copper Solutions Introduced Through the Roots

As above explained, the grape and the apple were chosen throughout the investigations recorded in this paper for the purpose of comparison with the peach in their behavior towards copper; the object being especially to select plants whose foliage is not injured to any appreciable extent in the practical application of fungicides. Moreover, since the question of leaf-fall must necessarily come into consideration here, comparative studies with the peach must be made on trees or shrubs, which annually lose their leaves when the plant enters the dormant, or winter, condition.

In the experiments discussed in this chapter the copper was supplied in the form of the sulphate, because this salt may be more accurately weighed in its crystalline condition than many other salts of this metal, and its solutions do not very soon deposit on standing. For the purposes of this investigation it appeared useless to employ copper in any other form. It has long been a sort of working principle in toxicology that the toxicity of a salt in solution is equal to the combined toxicity of its component acid and base,* or, as Kahlenberg and True† have shown for such solutions as are here considered, to the combined action of its ions. Coupin‡ has shown directly with the metal in question, that at the high degree of dilution sufficient to kill wheat seedlings the toxic action of the radical in solutions of various copper salts may be left out of consideration.

It is to be remembered that the writer's purpose here was simply to determine the relative susceptibility of the apple, peach, and grape to the poisonous action of copper. The water culture method was chosen as the only available one in this case. The method described by Heald,§ where the cessation of growth or appearance of death in the radicle of a seedling determines the fatal strength of the solution in question, is beyond a doubt far more delicate than the method here used, but after several attempts to germinate peach seed on blotting paper, etc., it was quite apparent that this method could not be used with the peach, on account of its capricious germination, to say nothing of the attacks upon it of damping-off fungi, etc. The grape seed were also found rather slow of germination. A number of trials were also made with cuttings of fresh growing shoots of the plants in question, but these gave no definite results and are not reported here.

A preliminary test was made in the autumn of 1900. Seedlings of the apple and peach and cuttings of grape were taken, which had grown as water cultures since about June 1 of same year. Apple seeds chiefly from

*Cf. Overton in reprint from Zeits. physikal. Chem., Bd. 22, 1897, p. 1.

‡Bot. Gaz., vol. 22, 1896, pp. 81–124.

†Comptes rendus Acad. Paris, t. 127, 1898, p. 400.

§Bot. Gaz., vol. 22, 1896, pp. 126–130.

Baldwin fruit were planted in sand and transferred to culture fluid (Knop's solution) after germination. Peach seedlings from native Tennessee seed were taken from nursery rows after having attained a height of about 15 centimeters, thoroughly washed, and transferred to culture fluid. Rooted grape cuttings of similar size and development were taken from soil and similarly treated. These cultures received the general treatment advised by Detmer.*

On October 8 two vigorous plants of each species were selected and placed in 0.00005 normal (gram molecules per liter of solution) copper sulphate solution.† On examination next day both apples were badly wilted; one peach wilted badly, the other slightly; the grapes showed no injury. On October 15, one week after beginning of experiment, both apples were entirely dead; the shoots of both peach plants had recovered their turgidity in the meantime, but some leaves had fallen off and their general appearance was not so healthy as check plants in distilled water; no difference could be detected between stem and leaves of grapes in copper solution and grapes in distilled water. The roots of both peach and grape were somewhat discolored, but were still turgid. About March 1 of the next year (1901) on removing plants from greenhouse (unheated during winter), it was found that both the grape and peach stems were still alive, although both had lost their leaves, as also check plants in the house had done.

A similar experiment was made in 1901. Peach seedlings were put into normal culture solution‡ on May 6; and grape and apple seedlings germinated in sand were transferred to culture solutions May 25. On July 16 there were selected for experiment 20 peaches, 10 apples, and 10 grapes. These plants, as in former experiment, were put one each into culture jars containing about 1800 cc of the solutions indicated in table below:

	Gram molecules $CuSO_4$ per liter of solution. (Fraction of "normal")
Peach, 4 plants	0.0001
Peach, 4 plants	0.000075
Peach, 4 plants	0.00005
Peach, 4 plants	0.00001
Peach, 4 plants	Distilled water
Apple, 2 plants	0.00006
Apple, 2 plants	0.00003
Apple, 2 plants	0.00001
Apple, 2 plants	0.0000075
Apple, 2 plants	Distilled water
Grape, 2 plants	0.0005
Grape, 2 plants	0.0001
Grape, 2 plants	0.00007
Grape, 2 plants	0.00004
Grape, 2 plants	Distilled water

*Das pflanzenphysiologische Praktikum, 1895, pp. 1–7.

†All the chemicals used in these investigations were "C. P." of standard manufacture (mostly Kahlbaum's) unless otherwise stated.

‡For formula see p. 39.

The plants were put into their respective solutions as shown in the table above about 6:00 p. m., July 16. From notes taken subsequently the following are given as bearing on the subject under discussion:

July 17—About 10:00 a. m.: All plants more or less wilted except checks in distilled water. At 6:00 p. m.: Peaches all recovered and of normal aspect; apples still wilted; grapes, 1 each of plant in 0.0005, 0.0001, and 0.00004 normal still wilted, others erect.

July 19—Apples in copper solutions all dead, except one plant in 0.00006 normal. This plant had a more vigorous root development than the others, and lived until July 21.

Photographs were made of these specimens on the afternoon of July 30 (see plate I).

July 31—Peaches: Dead throughout, one plant in 0.0001 normal, 3 plants in 0.000075 normal, 1 plant in 0.00005 normal. Stem still alive: 2 plants in 0.00005 normal. Terminal bud alive, leaves all dead: 3 plants in 0.0001 normal, 1 plant in 0.000075 normal, and 1 plant in 0.00005 normal. Bearing some healthy leaves: 4 plants in 0.00001 normal. In distilled water, still healthy. Roots of all the plants in copper solutions were more or less browned, but where stem was still alive roots also seemed to be living. (Fig. 1, plate I).

Apples: All dead 10 days since, except those in distilled water, which have continued to grow. (Fig. 2, plate I).

Grapes: Dead throughout, 1 plant in 0.0005 normal, 1 plant in 0.0001 normal, 1 plant in 0.00007 normal. Leaves dead, rest of plant alive: 1 plant in 0.0001 normal, 1 plant in 0.00004 normal. Bearing one or more living leaves: 1 plant in 0.0005 normal, 1 plant in 0.00007 normal, 1 plant in 0.00004 normal. In distilled water, all plants healthy. Here, as in case of peaches, roots appeared to be living where stem was still alive. (Fig. 3, plate I).

Before drawing any general conclusions from these experiments, two sources of error must be borne in mind. In the first place it was unfortunately necessary to use plants of different sizes* and of different stages of development, because, as above explained, the seeds of the three plants under consideration could not be made to germinate at the same time. As may be seen from plate I, the peach plants used were considerably larger than either the apple or the grape plants. Dehérain and Demoussy† found that the small quantities of copper present in water distilled from copper vessels will arrest the development of the roots of seedlings placed singly in vessels, whereas, if a number of such seedlings are placed together, in a limited quantity of this water, the injurious effect of the copper is not observed, because it is absorbed by all equally and by no one in toxic quantity. Clark found the same fact true of various fungus spores germinating in very dilute copper solutions.‡ It is probable, therefore, that smaller peach plants would have succumbed in a somewhat shorter time than the ones used in the experiments of 1901 above recorded.

*There was, however, but little difference in the relative dimensions of plants used in experiment in autumn 1900.

†Annales agronomiques, t. 27, 1901, pp. 553, 559.

‡Bot. Gaz., vol. 33, 1902, p. 28.

But since the quantity of solution accessible to each plant was relatively large (1800 cc), this probably did not materially affect the results.

In the second place, in an experiment made after this fashion the relative transpiration of leaves and absorptive action of roots must be taken into consideration, as well as the fact that dead roots may continue for a time to absorb water.*

As a general principle, it may be assumed that the cells of the various organs of a plant will have, at least so far as concerns the conditions here under consideration, the same relative sensitiveness to the poisonous action of the same substance.† There are some good reasons, however, for believing that the peach leaf is more sensitive to various poisons than the other organs of the plant, as will appear from evidence given in Chapter IV.

The following conclusion seems to be fully established by the foregoing experiments:

The roots of the apple are decidedly more sensitive to the toxic action of copper than are those of the peach, which latter are somewhat more sensitive to its action than those of the grape. The same relative sensitiveness to copper poison seems to hold true of the other organs of these plants, with the possible exception of the leaves of the peach.

*See e. g. Watson, Absorption of water by dead roots, Annals of Botany, vol. 8, 1894, pp. 119, 120.

†Compare, however, Pfeffer, Pflanzenphysiologie, Bd. II, pp. 343, 344.

CHAPTER II

Poisonous Action of Copper on the Leaves and Certain Conditions Affecting this Action

SOLUBLE COPPER SALTS

It has long been known that solutions of certain copper salts, as the sulphate for example, unless very dilute, injure the foliage of most plants ordinarily sprayed for fungous diseases.* Copper sulphate solution, for example, is considered a useful spore destroyer for the orchard and vineyard during the winter condition of the plant, but must be used with great caution on living leaves.

Two experiments were made with soluble copper salts on peach trees which have an important bearing on the main subject of this paper. Solutions were made in distilled water containing 0.05 per cent copper acetate, sulphate, chloride, and nitrate, and sprayed on the leaves, as explained below, by means of a carefully cleaned atomizer, so as to cover each set uniformly with a dew-like deposit. Four large seedling peach trees were chosen and one twig appropriately marked for each of the solutions in question on each of the trees, making four twigs sprayed with each solution. An application was made on the morning of July 23. No rain occurred during the experiment until the afternoon of July 28, when 0.01 inch (=0.25 mm) fell. An examination made on July 25 showed after two days no injury on any of the leaves sprayed with copper acetate; injury to only one of the twigs sprayed with the sulphate, as shown by red spots; serious injury to one twig sprayed with the chloride, spots on the other three; still greater injury in the case of all twigs sprayed with copper nitrate. A subsequent examination made on July 29 and 30 revealed almost without exception the same relative injury from the different solutions. On each tree at this date the acetate had produced scarcely any injury, while the sulphate had produced more in each case, the chloride still more, the nitrate most of all.

To these same trees were applied a number of other copper mixtures to be mentioned presently, the effect of which furnished an interesting study of individual or varietal susceptibility to copper poisoning. As will be explained later, copper hydrate was used as the basis of a number of other sprays applied to other twigs on these same trees at the same time. *Without exception the tree showing the greatest injury from these soluble copper salts also showed the greatest injury from copper hydrate.* It is a simple matter from notes taken at the time to arrange the trees in the order of their sensitiveness to the toxic action of copper. This experiment was repeated in essentially the same manner on four other trees on September

*Cf. Lodeman, The Spraying of Plants, p. 142; Hollrung, Chemische Mittel gegen Pflanzenkrankheiten, pp. 73 et seq.

14 and 15, except that the solutions contained equivalent amounts of copper. The same results were obtained, except that in this case the individuality of the trees was emphasized even more strongly than in the former experiment.

In the order of their injury to peach foliage these copper salts may very definitely be placed thus: (1) nitrate, (2) chloride, (3) sulphate, (4) acetate. In the degree of injury to the leaves they may also be placed in two distinct groups, the acetate and sulphate being decidedly less rapid in their destructive effects, the nitrate and chloride decidedly more so. For reasons set forth on p. 23 one would expect the toxic action of these various salts to be the same where solutions of equivalent strengths of copper were used, but the conditions are very different from those where solutions are applied to roots.*

It will be noted that the above results were obtained for the most part within five days after application of the spray and that during these five days no rain occurred. The weather was mostly clear and the leaves equally exposed to dew at night. That a different state of the weather would have produced quite different results is evident. The weather conditions were just such as to allow the presence on the leaves at night of solutions of the different salts of approximately the same concentration. The different degrees of solubility of these copper salts may doubtless account in part for the different toxic effects here noted, but it seems very much more reasonable to assume that an explanation for the marked difference in the injury produced on the one hand by the acetate and the sulphate, and on the other hand by the nitrate and the chloride, is to be found in the deliquescent property of the latter two salts. The nitrate and the chloride resting on the leaves in the form of minute crystals absorbed moisture from the rapidly cooling air towards evening and formed drops of a saturated solution even before the dew began to deposit. The acetate and the sulphate remained undissolved until the actual deposition of dew on the leaf.

From these considerations the following conclusions may be drawn, so far as the points in question relate to the peach leaf:

1 Among the factors influencing the injury of copper compounds sprayed on leaves, their solubility in water, and especially their deliquescence plays an important part. Other things being equal, a salt of copper having a tendency to remain on the leaf in a liquid condition for a greater part of the day will produce the greater injury.

2 There is a great difference in the susceptibility of individual trees to the poisonous action of copper, and those individuals more injured by soluble copper salts on their leaves also show greater injury from copper hydroxide, Bordeaux mixture, etc.

PURE BASIC COPPER

In the writer's former paper† it was shown that under orchard conditions copper hydroxide [$Cu(OH)_2$], copper-dicupric hydroxide [Cu_3

*Sturgis (Rep. Conn. Exp. Sta., 1900, p. 232) found normal copper acetate in very dilute solution to cause but slight injury to peach foliage.

†Bul. Tenn. Exp. Sta., vol. 8, No. 3, 1895, pp. 35–40.

$O_2(OH)_2 = Cu(OH)_2 + 2CuO$], copper carbonate and ammoniacal copper carbonate are decidedly more injurious to peach leaves than the Bordeaux mixture containing an excess of lime. Among other similar results may be mentioned those obtained by Lodeman,* who found ammoniacal copper carbonate more injurious to the foliage than the ordinary Bordeaux mixture, although the latter contains much more copper.

The writer has frequently within the past three years repeated this experiment in various ways and has uniformly obtained after the lapse of a greater or less time the same result, viz.: copper hydrate always injures peach foliage under normal conditions in far less time than does the Bordeaux mixture. During the summers of 1900 and 1901 some further experiments were made which may be described here. In the first place, it is necessary to explain somewhat in detail the method of preparing the substances in question.

Metallic copper: To a solution of copper sulphate in distilled water was added a piece of metallic zinc, "free of arsenic." The accumulated precipitate of metallic copper was washed first with dilute hydrochloric acid to remove any traces of zinc adhering and then repeatedly washed for some time with distilled water. There is thus obtained an impalpable powder of dark color and faint metallic lustre. Doubtless copper oxide is formed in the process, and the experiment cannot be worth much except by way of verification of other results. Used only qualitatively, a small amount being added to distilled water.

Copper oxide (CuO): Copper nitrate was kept at a red heat for about an hour in a porcelain dish. The resulting oxide was then repeatedly washed with distilled water in order to remove any traces of nitrate remaining. Applied in distilled water and used only qualitatively.

Copper hydroxide [$Cu(OH)_2$]:† Of copper sulphate 14.38 g were dissolved in distilled water, then an excess added of sodium hydroxide. To this was added a small quantity of ammonium chloride.‡ The resulting precipitate was poured on a filter and washed by means of a filter pump almost continuously eight to 10 hours a day for one week. The resultant filtrate was tested from time to time with silver nitrate, until even after remaining in contact with the precipitate over night it gave not the slightest reaction for chlorides. This precipitate was added to water sufficient to make one liter of the mixture. While a small quantity was lost in the process of washing, the copper hydroxide present was approximately equal to that in the Bordeaux mixture as commonly made in this country (6:4:50 formula). The mixture is of a somewhat more greenish tint than properly made Bordeaux mixture.

Copper-dicupric hydroxide [$Cu(OH)_2 + 2CuO$]: Made just as last, except that no ammonium chloride was used, and the precipitate was washed with boiling water, until, even after boiling down to small volume, the washings gave no precipitate with barium chloride. This mixture has a brownish black color, the precipitate in all respects appearing to the eye like copper oxide.

*Cornell Exp. Sta. Bul. 60, 1893, p. 300.

†See page 85.

‡Roscoe and Schorlemmer, Treatise on Chemistry, vol. 2, pt. I, p. 331.

An experiment was begun August 18, 1900 by spraying marked shoots on five peach trees with copper-dicupric hydroxide. On August 20, sets of twigs on same trees were sprayed with the mixture of metallic copper above mentioned, with copper oxide, and with Bordeaux. Within five days after application very serious injury appeared as the result of the action of copper-dicupric hydroxide on four of the trees, the fifth having sustained but little injury from any of the sprays by that time. Within eight days from date of application the metallic copper had produced greater injury than Bordeaux applied at the same time. Copper oxide had caused less injury than the metallic copper, evidently, as examination with a lens showed, because several showers occuring meantime had washed the powder off. Yet, considerable injury was shown on these leaves at the end of 10 days.*

At the same date as last these same mixtures were sprayed on apple and grape leaves in orchard and vineyard. Although they were watched for some days after the injury appeared on the peach leaves, no injury was observed from any of these mixtures, and on the strength of this observation it was stated by the writer in his preliminary report on these investigations† that copper hydroxide produces no injurious effects on apple and grape leaves. Observations made the next season go to prove this first conclusion incorrect. In July, 1901, a number of small drops of $Cu(OH)_2$, $Cu_3O_2(OH)_2$, and Bordeaux were placed on the upper surfaces of grape leaves in the vineyard in order to make some tests as to their effect on starch production (see p. 75). An examination of the grape leaves a few weeks later showed, after cleaning off the spots of $Cu(OH)_2$ and $Cu_3O_2(OH)_2$, very evident dark colored specks. On the older leaves these specks were confined almost exclusively to the area of the leaf directly under the spot. In younger ones specks were sometimes more or less scattered over the whole leaf surface, but bore such an evident relation of position to the spots of copper that no doubt could exist as to their cause. Sections were made through a number of these specks, which showed total disorganization of the contents of the palissade cells, extending in many cases into the cells of the spongy parenchyma underneath. These dead cells were in almost every instance located near the veins. During the time that had elapsed since the application of the copper heavy rains had occurred.

A similar experience was had later with apple leaves. A number of apple leaves on several different orchard trees received small drops of the above mentioned mixtures on August 24 and August 27, 1901. A number of these leaves were gathered on October 14 and carefully examined. The $Cu(OH)_2$ and $Cu_3O_2(OH)_2$ still remained, the spots being distinctly visible to the naked eye, while the Bordeaux had almost entirely disappeared. In almost every case dead and brown areas coextensive with the

*A phenomenon was observed here which illustrates in a striking manner how copper sprays may prevent fungus attacks on the fruit of grape, for example, even when they do not adhere to the fruit. A number of peach leaves on shoots lying directly beneath those sprayed with copper hydrate, etc., showed considerable injury, evidently caused by the copper washed down from above by rain or dew. Germinating fungus spores would of course be destroyed in the same manner.

†Science, N. S., vol. 14, 1901, pp. 221, 222.

copper spots were quite conspicuous, while the rest of the leaf still retained a healthy green color. While the Bordeaux spots were washed off, dead areas in the vicinity of their former location plainly indicated that this mixture too had produced some injury, though not so much as in the other two cases. There was considerable rainfall during the interval, the local weather record showing 6.71 inches (= 170.4 mm). The normal precipitation for this period is 4.63 inches (= 117.6 mm). Similar experience with fungicides on other plants than those with "delicate" foliage has been reported by various investigators. Lodeman* records an instance of injury to the fruit of apple and pear as a result of spraying with the Bordeaux mixture during a wet season. It is especially significant that Lodeman used the ferrocyanide test in preparing his Bordeaux mixture. This indicates that his mixture contained but a slight excess of lime, hence, after a few days' exposure to rains, almost pure copper hydroxide remained. Lodeman, however, seems to have observed no injury to the leaves in his experiments. Müller† found injury to apple foliage from various copper mixtures.

The localization of the injury produced by basic copper is of striking interest. The experiment last described shows the great durability of copper hydrate when precipitated from copper solutions and this mixture allowed to dry on the surface of a leaf. Of course, a shower falling before the evaporation of the water containing this precipitate washes it off; but being allowed to dry, it persistently adheres for a long time, though frequently exposed to hard rains. The fact that a deposit of the hydrate still remains visible after being exposed to varying weather conditions for over seven weeks is quite significant. Yet, the minute specks above referred to as occurring on grape leaves show that minute quantities of the copper are scattered over the leaf around the spots, but since these specks were so much more numerous immediately beneath the copper, the effect on the surrounding region may be left out of account. Some experiments on this local injurious action on the peach leaf proved very instructive.

The first externally visible injury resulting from the application of copper in any form to peach leaves is usually reddening of the tissues in the immediate vicinity of the copper. However, under certain conditions to be mentioned below, and under action of a very strong solution of a copper salt, this preliminary reddening does not occur, but the tissues turn brown in a short time, indicating in every instance rapidly fatal poisoning. In other instances, especially where the leaves are in a shaded situation and the mixture is uniformly spread over them, an entire leaf may gradually turn yellow and eventually drop off.‡

Usually within one or two days after the reddening effect the healing process discussed in Chapter IV sets in and the whole region concerned drops out (see Fig. 1, plate V). In other cases this reddening does not occur, and before any injury appears to the eye a considerable area separates by reason of the healing process and drops out. Both cases produce the so-

*Cornell Exp. Sta. Bul. 86, 1895, pp. 119, 120; also Garden and Forest, vol. 7, 1894, pp. 456, 457.

†Zeits. Pflanzenkr., Bd. 10, 1900, pp. 233, 311, 312.

‡Cf. Sturgis, Conn. Exp. Sta. Rep. 1900, p. 245.

called "shot-hole" effect. In the peach leaf, just as in the case of the grape leaf above mentioned, sections were made through regions in the incipient reddening stage. Here likewise the assimilating cells in close proximity to the veins show the injury first.

On August 18, 1900, on same five peach trees as used in experiment described on p. 30, 10 leaves on one shoot of each tree received small drops of cupric hydroxide applied to median region of the upper surface. Injury was shown in about 12 days by characteristic "shot-holes." In the great majority of cases these shot-holes appeared at least partly in contact with area covered by copper, though rarely, if ever, coextensive with the copper spot. This experiment alone suffices to disprove Sturgis'* supposition that the stomata are responsible for this injury to peach foliage. It is thus shown to be local in character, and independent of the stomata. There are no stomata on the upper surface of the leaf, and although as shown above the copper may be distributed to a slight extent from these local areas to other parts of the leaf, yet if it acted in any way through the stomata it would have to be carried around the margin of the leaf and stop directly under the spot on the upper surface—a supposition manifestly absurd. Moreover, it was shown above that exactly the same local injury occurs under certain conditions when drops of copper hydrate, etc., are applied to upper surface of apple and grape leaves. This does not indicate, however, that the injury may not be as great or greater on lower surface of leaf (Cf. p. 48).

On August 19, 1901, spots of $Cu(OH)_2$, $Cu_3O_2(OH)_2$, and Bordeaux mixture were applied in the usual manner to a number of leaves on the same seedling tree. They were placed on both upper and lower surfaces in regular order, some drops of all three mixtures being placed on each leaf, but on only one surface of the same leaf. A number of these leaves were gathered on August 24 five days later—and examined. In not a single instance was any injury evident in the neighborhood of the Bordeaux spots on either the upper or the lower surface. Numerous red spots and dead areas were visible under and around spots of $Cu_3O_2(OH)_2$ on the upper surface; below, it had not adhered and nothing could be determined in this case. About the $Cu(OH)_2$ areas on upper surface were also many red spots and brown areas, though not so numerous as in vicinity of $Cu_3O_2(OH)_2$; on lower surface in some cases was visible *no effect whatever, in others far more widespread injury than on upper surface.* An especially striking example was furnished by one leaf. The appearances here recorded were distinctly visible even in the dried specimen. Two spots of $Cu(OH)_2$ were placed one on either side of midrib, and nearly equidistant from base. Under one spot no discoloration whatever was visible; a little to one side of the other was a brown area of dead tissue about 2 mm in diameter. The neighboring vein showed a deep purple coloration all the way to the midrib, a distance of 5 mm, and all the veinlets for several millimeters around appeared slightly purple. Not only this, but three secondary veins above the spot for nearly 3 centimeters showed the same characteristic purple stain, which also affected the midrib and many of the intervening minor veinlets; in several cases appeared also

*L. c., pp. 249, 250.

brown areas nearly as large as the original one under the copper spot. The weather record from August 19 to August 24 is as follows:

Date	Rainfall—inches
August 19	0.01
August 20	0.07
August 21	0.20
August 22	0.12
August 23	0.01
August 24	0.05
Total	0.55 (= 13.9 mm)

The experiment was repeated August 24. On August 28 no injury whatever was apparent under any of the spots of Bordeaux, either above or below. In scarcely a single instance was any injury visible on this date from either the $Cu(OH)_2$ or the $Cu_3O_2(OH)_2$ applied to upper surface; but over every spot of $Cu_3O_2(OH)_2$ applied to lower surface an intensely red coloration of the tissues had occurred. This reddening was also visible over the spots of $Cu(OH)_2$, but by no means so intense as over those of $Cu_3O_2(OH)_2$. The weather record during this interim is as follows:

Date	Rainfall—inches
August 24	0.05
August 25	0.01
August 26	0.00
August 27	0.92
August 28	0.03
Total	1.01 (= 25.6 mm)

Duration of rainfall in daytime 55 min.

A full discussion of the results obtained in these two experiments requires weather conditions to be taken into account, and especial attention is given to this on p. 48.

One or two additional phases of the local action of basic copper on peach leaves demand consideration here. The statement was made above that the dead or discolored areas are rarely coextensive with the spots of $Cu(OH)_2$ or $Cu_3O_2(OH)_2$. Fig. 2, plate VII shows at *a* and *b* small dead areas directly beneath spot of $Cu_3O_2(OH)_2$. The unshaded area indicates almost exactly the outline of the space covered by copper, except the starch ring around the dead areas *a* and *b* (see p. 77). This very distinctly indicates that there are special points on the surface of the leaf where the injury is more marked than at others, although the whole surface be uniformly covered with the copper.

The frequent appearance of red spots and shot-holes around these areas of copper hydrate, etc., as well as beneath them is very evidently connected with weather conditions. In the first experiment above referred to, for example, where drops of $Cu(OH)_2$ and $Cu_3O_2(OH)_2$ were applied to upper surfaces of peach leaves on August 19, these red spots and even small brown areas were quite conspicuous but rarely one centimeter from the copper. A glance at Fig. 2, plate VII, where the proportion of

starch surrounding a spot of $Cu_3O_2(OH)_2$ is accurately indicated by the intensity of shading, gives a very accurate idea also of the distribution of red spots as they might have appeared a few days later on this same leaf. We have, therefore, a condition here exactly like that mentioned on page 30 as occurring on grape leaves.

The healing of a local injury caused by copper is discussed on p. 69. Attention is here called to a quite frequent phenomenon, of which an adequate conception may be had by inspecting the photomicrograph Fig. 1, plate V. The small dark spot in the center indicates the first region killed by $Cu_3O_2(OH)_2$. Around this spot healing and exfoliation occurred, followed by one or two ineffectual attempts to repeat the process on a somewhat larger scale, until finally the whole area was sloughed out as shown by the irregular white band surrounding. All this occurred within 18 days after application, and merely serves to emphasize the fact that there are minute areas of the leaf more subject to copper injury than the general surface.

Sturgis* has called attention to the more frequent occurrence of these injuries near the margin of the leaf. The writer can corroborate this observation. It is especially observable when peach leaves in a saturated atmosphere are sprayed with copper hydrate, in which case this marginal injury is practically all that may be seen. It is also quite conspicuous in the photograph shown in plate III. The marginal glands must play an important rôle here and the subject is further discussed on page 53.

Attention may here be especially called to the difference in local injury between $Cu(OH)_2$, etc., and Bordeaux mixture when applied to peach leaves as a spray under normal conditions. *Where Bordeaux spots are distinctly visible,* caused from evident accumulation and subsequent evaporation of larger drops, *there is rarely if ever a local injury; but pure copper hydrate and copper-dicupric hydroxide always cause a more or less local injury.*

The subject of leaf-fall is discussed in chapter IV, but one more point must be mentioned here as characterizing local and general action of copper on peach leaves. It will be shown later (p. 72) that the leaves in question are especially sensitive to various agencies producing normal leaf-fall. An extensive injury of any character, as the mechanical removal of most of the blade, causes formation of a normal absciss layer and consequent dropping of the leaf. In like manner, as shown by Duggar† the peach leaf very quickly rids itself of an injured or dead area. For this reason, the application of strong solutions of copper salts, such as the sulphate, nitrate, etc., under certain conditions appears to produce less injury than copper hydroxide. The drops of copper solutions act very rapidly locally, soon kill these areas, and the leaf rids itself of the dead tissues in the manner above referred to. Rains and heavy dews contribute to this effect by washing off the soluble copper scattered over the leaf, injury then occurring only at the "sensitive points" above referred to. If this injury is not too widespread the leaf rids itself of all the region at-

*L. c., p. 245.

†Proc. 19th Ann. Meeting Soc. Prom. Agr Sci., 1898.

tacked by copper and the remaining portion of the blade survives and continues to assimilate. If, however, the more difficultly soluble and more slowly acting copper hydrate covers a large part of the leaf's surface, it may fall off entire even before very great local injury manifests itself. In this way, no doubt, are to be explained Sturgis'* results with basic copper acetate, which he found to cause more injury to the leaves of the peach than the normal acetate. Therefore, in making comparative estimates of the injury of different copper compounds on peach foliage the fact just mentioned must be taken into consideration. The relative time required for the appearance of the reddening or browning effect is, probably, the most trustworthy indication of the comparative injury apparent to the eye. This fact was given due consideration in all the experiments described above. For a very similar reason copper hydrate, etc., may often appear to be more injurious to old than to young foliage, an erroneous conclusion reached by Sturgis.†

Finally, it may here be stated that the very young leaves at the ends of growing peach shoots always suffer great injury within twenty-four hours after being sprayed with basic copper in any form and even with Bordeaux mixture. A complete covering of freshly slaked lime failed to produce any apparent injury, hence it may be definitely stated that copper is alone responsible for this effect. So fatal to the very young leaves at the ends of shoots is this action, especially early in the season, that peculiar deformed leaves often mark very definitely the growth of shoots during the interval of time between sprayings. The portions of these leaves covered by the mixture are killed outright, hence this is, so far as its injurious effects go, a purely local action. The attachment of the dead, shriveled tissue to the rapidly growing lamina causes the living portion to assume distorted forms. This extreme susceptibility of young leaves to injury seems to vanish about the time of the disappearance of the so-called "varnish"‡ from their surfaces. This matter is again referred to on p. 53.

With regard to the action of basic copper on leaves the following conclusions seem to be justified from the above considerations:

1 Basic copper in the form of the finely divided metal, the oxide, the hydroxide, the dicupric hydroxide, or the ammoniacal copper carbonate, applied as a spray, is very much more injurious to peach leaves, and somewhat more injurious to apple and grape leaves than properly made Bordeaux mixture.

2 The injury of copper in any of these forms is much more rapid on young than on mature leaves.

3 Copper injury on leaves is manifested externally in three different ways: (1) very rapid action turns the whole affected tissue of the leaf brown; (2) slower action on the peach results sometimes in reddening

*L. c., pp. 231, 232.

†L. c. There may, however, be some tendency in this direction, for Clark (l. c., p. 34) has called attention to the fact that when rapid growth is in progress a greater absolute quantity of a poison is required to produce fatal results. Wille (l. c., p. 321) also found that salts are more readily taken up by leaves beginning to fade.

‡Kerner and Oliver, The Natural History of Plants, vol. I, p. 313.

the contents of the cells immediately under the copper, or more seldom in the exfoliation of the tissues concerned without previous reddening; on grape leaves the injury is marked by simple purplish coloration of smaller scattered groups of assimilative cells; (3) very slow action on peach leaves may result, especially in shaded positions, in the fading and subsequent dropping of the whole leaf. The appearance of the injurious effects is the same whether soluble copper salts or basic copper are applied.

4 Copper hydroxide and copper-dicupric hydroxide will persist on leaves exposed to weather longer than the Bordeaux mixture.

5 Isolated drops of copper hydroxide and dicupric hydroxide suspended in water on surfaces of leaves and allowed to dry up leave deposits that endure for a long time, but are slowly broken up by rain or dew and scattered over a limited area, whether as a result of solution or of mechanical disintegration does not appear from the foregoing experiments.

6 The injury produced by spots of pure basic copper on leaves is local in its character, although not often coextensive with the copper spot. When this local injury occurs at any appreciable distance from the spot it indicates more susceptible areas of the leaf, the copper reaching this area through drops of dew or rain on the surface of the leaf. From these susceptible points the injury invades progressively the surrounding region, causing concentric zones of exfoliation due to the healing process.

7 The stomata are not responsible for the injury of fungicides to peach foliage. Copper hydroxide applied to the lower surface of the leaf sometimes produces less injury than on upper surface, at other times very much more, depending on certain weather conditions.*

8 The injury of pure basic copper to grape and peach leaves begins in the cells in close proximity to the veins. Sometimes copper hydroxide injuries to lower surface may be traced along the veins for some distance from the copper covered surface, taking thus the same course as that taken by a salt in solution.

9 The injuries caused by Bordeaux mixture applied as a spray on peach leaves are rarely located under the region where the spots of the mixture are distinctly visible, but occur, as a rule, between these spots.

10 Local injuries are more frequent in the marginal than in the median region of the peach leaf.

11 In judging of the injurious effects of different copper sprays on peach leaves under orchard conditions, the relative loss of leaves is not always a correct measure of the injurious character of a copper combination; especially is this true where the solubility and adhesive properties of the mixtures after drying differ to any extent.

EFFECT OF VARIOUS ADMIXTURES WITH BASIC COPPER

It now seems pretty generally agreed that in a properly made Bordeaux mixture the copper exists only as the normal cupric hydroxide,† the reaction occurring in the preparation of the mixture being represented by the following equation: $CuSO_4 + Ca(OH)_2 = CaSO_4 + Cu(OH)_2$. As

*See page 48.

†Cf. Aderhold, Centralbl. Bakteriol., Bd. 5, 1899, p. 218; Rumm, Beiträge zur wiss. Bot., Bd. 1, 1897, p. 81; Clark, Bot. Gaz., vol. 33, 1902, p. 41.

ordinarily made the mixture contains a large excess of $Ca(OH)_2$, which on exposure to the atmosphere with a greater or less lapse of time combines with the atmospheric CO_2 to form $CaCO_3$. The length of time required for completion of this latter reaction seems to differ according to conditions. Millardet states* that this reaction occurs within a "few days."

The writer made an experiment by leaving ordinary Bordeaux mixture exposed on a glass plate out doors and testing with phenolphthalein to determine when alkalinity was neutralized. No coloration resulted after one night's exposure to dew. In any case it may be assumed that almost from the instant when Bordeaux mixture is applied to foliage the following compounds occur in it: copper hydroxide, calcium hydroxide, calcium carbonate, and calcium sulphate, together with traces of any impurities that may be present in any of the raw materials used. Among the latter Aderhold† has given especial attention to iron. The $Ca(OH)_2$, as stated, is soon converted into the carbonate.

In the writer's first paper on this subject‡ careful experiments were made on twigs in orchard to determine effect of various combinations of these substances when applied to peach trees as a spray. Among other conclusions there reached the following may be quoted: "The injury caused by copper hydroxide, like that caused by the Bordeaux mixture, may be greatly reduced by adding milk of lime .... but the sulphate of lime will not diminish the injury." These experiments were carefully repeated last year and additional ones made, which unmistakably confirm the results before published. Two shoots each on two seedling peach trees were sprayed June 25, (1) with the same copper-dicupric hydroxide mixture mentioned on p. 29, (2) copper hydroxide with milk of lime added, (3) copper hydroxide with calcium carbonate added, (4) same with calcium sulphate added, (5) ordinary Bordeaux mixture containing sufficient excess of lime to cause pellicle of carbonate of lime on the surface when breathed upon. Enough of each lime compound in question was added to give a distinctly milky color to the mixture. The carbonate and sulphate of lime were "chemically pure;" the hydrate was prepared from ordinary commercial quicklime. In order to determine definitely the effect of the presence of the sulphate and carbonate with copper hydrate on the leaf it was necessary to add these compounds occasionally as a spray in distilled water; for rains will readily dissolve off the sulphate, and wash off the carbonate when applied as such simply suspended in water. For reasons which will appear later (p. 45) distilled water was sprayed on the other experimental shoots each time when fresh application of $Ca(OH)_2$, $CaCO_3$, and $CaSO_4$ was found necessary. It was thus found necessary to make only one additional spraying of $Ca(OH)_2$ and $CaSO_4$, while two were made of $CaCO_3$, before photographing on July 12. The contrast was very striking as between the effect of the $Cu_3O_2(OH)_2$, $Cu_3O_2(OH)_2 + CaSO_4$, and Bordeaux, on the one hand, and the $Cu_3O_2(OH)_2 + Ca(OH)_2$, and $Cu_3O_2(OH)_2 + CaCO_3$ on the other. Without going

*Quoted by Viala, Les maladies de la vigne, 1893, p. 132. Other authorities place the time necessary to complete the reaction at one to 10 days (Zeits. Pflanzenkr., Bd. 9 1899, p. 349).

†[illegible]

‡Tenn. Exp. Sta. Bul. 3, vol. 8, 1895, pp. 39, 40

into further details than to state that on both experimental trees the hydroxide and the carbonate of lime were equally effective in preventing the injury, the reader is referred to Fig. 1, plate II. This figure represents all the twigs in question carefully removed from one of the trees and photographed July 12. The contrast was equally striking on the other tree. This experiment decides definitely a very important practical question. *The injury of the Bordeaux mixture to peach foliage does not result merely from the neutralization of the caustic lime by the CO_2 of the atmosphere,* for calcium carbonate, the resulting compound, retards the injury just as well if kept constantly on the leaves. This experiment was repeated, as will be shown later (p. 40) on four other trees with essentially the same results.

An equally interesting and instructive experiment was made on four orchard trees, two of which were sprayed on June 29 with ordinary Bordeaux (6 lbs. copper sulphate to 50 gallons water), the other two with a similar mixture containing half as much copper sulphate ("half strength" Bordeaux). Freshly prepared milk of lime was added to the copper sulphate solution until a distinct pellicle formed on breathing on the surface. Both mixtures thus contained when applied a considerable excess of lime, as was apparent even from their color. On the following dates, determined by the frequency of rains, one-half of the entire crown of each tree was sprayed with thin milk of lime, care being taken to avoid the application of any lime to the other half: June 29, July 5, July 17, and August 6. On August 15 one tree from each set received an application of lime, the design here being to determine whether frequent and long continued applications of lime would affect the result. On July 27 these trees were photographed, one of which is shown on plate VIII. Within less than two weeks after the application of Bordeaux mixture a very striking contrast was evident between the portion of the tree sprayed with lime and that not receiving this subsequent spraying. The leaves of the former at quite a distance revealed a healthy dark green color, while those on the latter appeared sickly and yellowish and soon began to fall off. The leaves that had been sprayed with lime remained on the trees as long as the leaves on neighboring unsprayed trees, while the portions of these trees that had not received the subsequent applications of lime had been almost completely defoliated long before the end of the season. The leaves sprayed with half-strength Bordeaux showed in some cases even greater injury than those sprayed with the mixture of full strength.

The part played by lime in the Bordeaux mixture has been a matter of considerable conjecture. Harrison* concluded from his experiments that lime must do more than merely to precipitate the copper from copper sulphate and render it insoluble. He conjectured that it might be taken up by the leaves and contribute to their nutrition, founding his assumption on the experiments of Boussingault† who showed that sulphate of lime, etc., may under certain conditions be absorbed by the leaf. Frank and Krüger‡ found spraying with lime to increase slightly the amount of

*Ann. Rep. Ont. Agr. Coll. and Exp. Farm, 1897, p. 128.

†Ann. de chim. et de phys., sér. V, t. 13, 1878, pp. 393, 394.

‡Arb. Deutsch. Landw.-Ges., Heft 2, 1894, p. 3.

starch elaborated in potato leaves, and Aderhold's* investigations to decide the question as to whether leaves sprayed with sulphate of lime may absorb enough lime to play any part as a nutrient were inconclusive, as is shown on p. 66. In consideration of the important rôle which lime plays in the metabolism of leaves and the well known fact that any well nourished cell can withstand extreme influences of any character better than one not receiving sufficient food, the possibility existed that the leaves sprayed with excess of lime might owe their immunity against injury by copper to the absorption of lime. The fact that the more soluble lime compound existing in the Bordeaux mixture, the sulphate, must soon be washed off by rain and dew, did not of itself especially militate against this possibility, for the carbonate of lime which remains on the leaf is soluble in water containing CO_2, and the carbon used up in the normal process of photosynthesis† passes the leaf cuticle as an aqueous solution of this gas. It was readily conceivable that this aqueous solution of CO_2 might carry with it some carbonate of lime into the assimilative cells of the leaf. Moreover, the investigations of Rumm and Frank and Krüger had shown that the assimilative process in the leaf is accelerated under the stimulant action of copper; this increased activity might demand a greater supply of mineral nutriment than the plant could obtain from the soil. It would in that case be a simple matter to supply lime to the soil, thus not only benefiting the plant as an ordinary fertilizer, but at the same time rendering its foliage immune from the injurious action of fungicides. For this reason the writer was led to make some experiments which show that the lime does not prevent the injurious action of copper on peach foliage by reason of its nutrient function.

In the first place, the above mentioned experiment with $CaSO_4$ indicates as much. Boussingault‡ found sulphate of lime to be taken up very readily by the leaf under certain conditions, and if the lime acted merely the part of a nutrient, the sulphate should prevent the injury by copper even more effectually than the far less soluble carbonate. Yet the sulphate is of no avail in this direction. It will also be shown presently that the presence of $Ca(NO_3)_2$, a salt whose base and radical are both important nutrients, increases very materially the injury caused by copper hydrate.

Several experiments were made with the direct object of deciding this question. On June 24, 1901, were chosen from a number of cultures four peach seedlings that had been growing in a normal solution§ since May 6, and four others that had grown since the same date alongside the former in Schimper's‖ solution poor in lime.¶ The latter set had already some days before begun to show very evident deterioration from lack of lime. Two of each set were sprayed with Bordeaux mixture and the other two with

*L. c., pp. 255, 256.

†Pfeffer, Pflanzenphysiologie, Bd. I, 1897, p. 167.

‡L. c., pp. 393, 394.

§Formula: $Ca(NO_3)_2$, 1.00 g; KNO_3, 0.25 g; $MgSO_4$, 0.25 g; K_2HPO_4, 0.25 g; NaCl, 0.25 g; Fe_2Cl_6, trace; H_2O, 1000 cc.

‖Flora, Bd. 73, 1890, p. 221.

¶Formula: $Ca(NO_3)_2$, 0.25 g; KNO_3, 1.00 g; $MgSO_4$, 0.25 g; K_2HPO_4, 0.25 g; NaCl, 0.25 g; Fe_2Cl_6, trace; H_2O, 1000 cc.

copper hydroxide, the jars being placed at once under the wire enclosure described on page 44, and thus exposed to dew and rain. In five days injury began to appear on plants sprayed with $Cu(OH)_2$ alike in normal solution and in solution poor in lime. Notes made on July 31 record even greater injury from both Bordeaux and $Cu(OH)_2$ on plants in normal solution than on those in solution poor in lime.*

Another experiment even more convincing was made. On the morning of July 22, 1901, one selected twig on each of four seedling peach trees was sprayed with the following: copper hydroxide, copper-dicupric hydroxide, copper hydroxide followed in a few hours with 0.5 per cent calcium hydroxide, $Cu(OH)_2$ with $CaCO_3$, $Cu(OH)_2$ with $BaCO_3$, $Cu(OH)_2$ with $SrCO_3$, and $Cu(OH)_2$ with $MgCO_3$, all mixtures of same strength. At the same time a similar set of twigs on the same trees were sprayed respectively with $BaCO_3$, $SrCO_3$, and $MgCO_3$, in order to determine whether these substances have in themselves an injurious effect when sprayed on the leaves. The injury began to make its appearance on the twigs sprayed with $Cu(OH)_2$ and $Cu_3O_2(OH)_2$ within three days. On the leaves sprayed with the pure carbonates of magnesium, barium, and strontium no injury ever occurred. These carbonates, having been applied in the form of fine pulverulent precipitates, were of course easily washed off by several intervening showers, and were renewed in form of spray on August 1 and August 7. Record made on August 14 shows decidedly less injury to foliage where the carbonates in question accompanied the copper than where the copper was applied pure, in spite of the fact that 4.68 inches (= 118.9 mm) of rain had fallen in the meantime. Here, as usual, the twigs sprayed with $Cu(OH)_2 + Ca(OH)_2$ held out longer than any of the others sprayed with copper, plainly because the lime thus applied adheres much better than any of the other compounds accompanying the copper. The experiment was repeated with even more striking results. Four trees like the last were treated in a manner quite similar to that above described. Here 10 leaves were marked off on each twig and sprayed as above explained on September 11, 1901. Fig. 2, plate II, shows the condition of the sprayed shoots on one of these trees on September 23. For this cut the twigs were very carefully removed and photographed on the last mentioned date. The contrast was equally striking on the other three trees, and photographs were made of two of them, but in order to get the contrast showing the effect of the carbonates in question in reducing the copper injury it was necessary to photograph the specimens at different dates. One tree was photographed September 18, another September 20, while the last, the one from which the cut was made, was photographed September 23, thus showing in a quite striking manner the individual susceptibility of trees above referred to (p. 28).

It thus becomes clear that the excess of lime in the Bordeaux mixture does not prevent the injury of the copper contained therein by reason of its nutrient function on the interior of the leaf. Barium and strontium are active poisons to the vegetable organism, as is also magnesium except in

*A striking fact to be mentioned later (p. 66) in reference to all these water cultures is that they suffered less injury from the copper than similar potted plants growing alongside.

small doses,* but their toxicity is so small as compared with that of copper that it plays no appreciable part in the case here under consideration. The effect of the carbonates of the alkaline earths in retarding the action of copper hydrate on the leaves is, however, unquestionable from the evidence just presented. Attention here is also called to some field experiments described on p. 38.

Experiments similar to the two last were carried out with solutions of the carbonates of sodium, potassium, lithium, and ammonium. The latter seemed to retard the injury a little, but not to any marked extent. Nothing could be concluded as to the effect of the carbonates of sodium, potassium, and lithium applied with the copper, because at the concentration used (0.5 per cent) as much injury resulted from the pure salts as when they were applied with copper. An interesting fact was observed as to the action of $LiCO_3$, namely, that the dead spots produced by it were much more abundant in the marginal region of the leaf. (See p. 53).

When some preliminary experiments gave results like those just explained, showing the influence of admixtures of carbonates of the alkaline earths on the injurious action of copper hydrate on peach foliage, it occurred to the writer desirable to know something as to the influence of various other substances on this injurious action. In addition to the various salts mentioned below, glucose, sucrose, and some other substances were tested. It is a common custom in Europe especially† to add sugar to the Bordeaux mixture; and, as is shown on p. 53, there are special secretions of grape sugar and gum present on peach leaves, which might play some part in the matter here under consideration.

In a preliminary experiment begun June 25, 1901, the injurious influence of the nitrate and chloride of lime when applied with $Cu_8O_2(OH)_2$ as a spray was clearly indicated. For experimental purposes two seedling peach trees growing under normal conditions were used. At the same time two potted seedlings of this year were sprayed with each of the mixtures described below and placed inside the greenhouse, two others similarly treated being placed outside, and thus exposed to dew and rain. This experiment has already been partly described on p. 37 merely to show the effect of the different constituents of the Bordeaux mixture. It is here again repeated merely to show the relative differences between the sulphate of lime on the one hand, as compared with the nitrate and chloride on the other when applied separately with basic copper. For the sake of brevity the notes taken on the shoots on trees on June 26 and June 29 and on the potted plants June 29 and July 10 are here tabulated.

*Loew, Physiological rôle of mineral nutrients, Bul. No. 18, Div. Veg. Phys. and Path., U. S. Dept. of Agr., 1899, pp. 38-44.

†Hollrung, Chemische Mittel gegen Pflanzenkrankheiten, 1898, pp. 104, 105.

Four peach shoots on two trees, sprayed first on June 25 and again on June 26. No rain.

Mixture Applied as Spray	Condition June 26	Condition June 29
$Cu_3O_2(OH)_2$	No injury	Numerous spots
" + $Ca(NO_3)_2$	Red spots	Much worse than last
" + $CaCl_2$	"	Still worse
" + $CaSO_4$	No injury	Numerous spots
" + $Ca(OH)_2$	"	No injury
" + $CaCO_3$	"	"

Two potted peach seedlings for each mixture, sprayed June 24-5; no subsequent spraying. Rainfall from June 29 to July 7, 0.50 inches (= 12.7 mm), no rain on other dates.

Mixture Applied as Spray	Condition June 29		Condition July 10	
	Plants Inside	Plants Outside	Plants Inside	Plants Outside
$Cu_3O_2(OH)_2$	No injury	A few red spots	No injury	Badly spotted
" + $Ca(NO_3)_2$	A few red spots	" "	Numerous sp's	" "
" + $CaCl_2$	Frequent red spots	Numerous red spots	Many spots & shot-holes	Worse than above
" + $CaSO_4$	No injury	No apparent injury	No injury	Spotted
" + $Ca(OH)_2$	"	No injury	"	No injury
" + $CaCO_3$	"	"	"	A few spots

From the above table it appears with striking emphasis that the addition of calcium nitrate and calcium chloride increased the injury of copper-dicupric hydroxide on the leaves of the tree. The effect of rain in this case may be fully appreciated if one imagines a shower to have fallen a few minutes after drying of the spray; all of the extremely soluble and deliquescent salts would have been at once washed off. Between the dates of notes on the tree shoots no rain fell; hence this experiment may be taken as a reliable indication of the effect of the constant presence of calcium nitrate and chloride with the basic copper on the leaf. These salts unquestionably increase the injurious action of the copper on leaves under normal conditions.

The part played by these deliquescent salts on leaves within and without the greenhouse affords an interesting comparative study. Protected from rain and dew not only did copper-dicupric hydroxide produce no injury when applied with the carbonate and hydroxide of lime, but even alone and with the sulphate no injury was produced. Under shelter in presence of the nitrate and chloride of lime, however, distinct injury was shown within four days. The potted plants outside verify the results obtained on the trees; viz., the nitrate and chloride of lime increase the injury caused by $Cu_3O_2(OH)_2$, the sulphate has no influence on this injury, while the hydrate and carbonate either prevent it altogether or greatly retard it. It also appears from this experiment, as is shown more definitely on p. 46, that even pure basic copper is without injury to peach leaves when they are protected from rain and dew.

Another experiment was begun on July 22, 1901, on a larger scale and with a number of other substances in order to determine their effect when mixed with basic copper and applied to peach foliage. Owing to the abundant rains soon following this first spraying with copper hydroxide,

etc., the effect of the soluble substances used could not be very decisive, and only those which gave unmistakable results are reported here. It must be borne in mind that in order to test thoroughly the effect of any soluble or non-adhesive substance under these conditions it is necessary to renew it frequently on the leaves if there is much precipitation of water either as rain or dew.

In this second experiment essentially the same methods were used as above described. Four sets of twigs were used on five different seedling peach trees, one set being divided between two trees because a fourth tree large enough to supply sufficient comparable twigs for the various tests was not available. The distribution of sprayed twigs on this tree was such as to test the comparative effects which it was desired to test in each case. The $Cu(OH)_2$, $Cu_3O_2(OH)_2$ and Bordeaux were applied first merely in distilled water, in every case the admixture in question being applied at once or a few hours afterward. Instead of entering into details a mere summary is given of the effect of these various substances on the injurious action of copper hydrate. Solutions 0.5 per cent strength were used, in most cases of C. P. materials. Sprays first applied July 22 and 23, 1901. Causing very decided increase in injury: sodium chloride, magnesium nitrate. Appreciably greater injury, but not so marked as the last: potassium nitrate, magnesium sulphate, potassium sulphate. Slightly greater injury: sucrose, glucose. Without effect on injurious action: tri-potassium phosphate, calcium oxalate, tri-calcium phosphate, dextrine, gum arabic. Slightly less injury: ammonium carbonate. Decidedly less injury: mono-potassium phosphate. To this list may be added the gum secretion of peach leaves (see p. 53), which was later found not to increase the injury in question. None of these substances produced injury when applied separately in 0.5 per cent solutions.

With regard to the effect of admixtures with basic copper applied to peach leaves, the following conclusions may be drawn:

1 Of the by-products of the Bordeaux mixture, calcium hydroxide and calcium carbonate tend to prevent the injury caused to foliage, while calcium sulphate is without effect on this injurious action.

2 The injury resulting from copper hydroxide or Bordeaux mixture may be greatly reduced by keeping constantly present on the leaves an excess of calcium hydroxide or calcium carbonate.

3 The action of this excess of lime in preventing the copper injury is not due to the nutritive function of lime absorbed by the leaves.

4 The carbonates of the alkaline earths in general are as effective as carbonate of lime in preventing this injury.

5 Some deliquescent salts [as $CaCl_2$ and $Ca(NO_3)_2$] very materially increase the injury of copper hydroxide to leaves; others (K_3PO_4) do not produce this effect. This action results under certain orchard conditions, and is especially marked in the case of sheltered leaves in the greenhouse.

6 Of certain mineral salts tested some increase the injury of the copper to a marked extent [NaCl, $Mg(NO_3)_2$], others to a less extent (KNO_3, etc.), others are indifferent in their action [$Ca_3(PO_4)_2$, etc.], while still others (KH_2PO_4) seem to diminish this injury.

7 Both cane and grape sugar increase the action of the copper slightly, while gum arabic and the gum secreted by peach leaves are without influence on this injury.

EFFECT OF RAIN, DEW, SUNLIGHT, AND HYGROSCOPIC MOISTURE

In the previous part of this discussion frequent reference has been made to the effect of rain and dew on the injury produced by fungicides. So far as known to the writer, no experiments have been made with the direct object of testing the effect of weather conditions on the action of the fungicide on the host plant. Clark* has recently clearly stated the logical deductions following his experiments on the toxic action of copper hydroxide on fungi, but made no direct experiments to test the various points here discussed; moreover, the writer† had already several months before published the general results of the experiments here given in detail.‡ Several writers however have suspected the part played by rain in the injury of fungicides to foliage, among whom may be especially mentioned Lodeman.§ The investigations hitherto made seem to have been directed chiefly if not exclusively toward the mechanical effect of falling rain on the adhesive properties of the mixture, although, as a matter of course, it has been recognized in all investigations as to the fungicidal action of Bordeaux mixture, etc., that the copper must act in the presence of rain or dew upon the leaves, since the presence of water is necessary for the germination of fungus spores. As to the direct stimulant action of copper on the host plant, resulting in the increased activity of the assimilative functions, etc., these various factors seem to have been entirely ignored.‖ There has been a considerable number of investigations on the adhesive properties of spraying mixtures applied to foliage and on their permanence under ordinary weather conditions. In this connection may be mentioned especially those of Millardet, Girard, Gouillon and Gouirand, and Stubenrauch.¶.

*Bot. Gaz., vol. 33, 1902, p. 44.

†Science, N. S., vol. 14, 1901, pp. 221, 222.

‡The writer showed priority of publication on these points in Bot. Gaz , vol. 33, 1902, pp. 244, 245, where may be found also Mr Clark's reply. Clark is, however, so far as may be judged from the review of Barth's paper (Bot. Centralbl., Bd. 61, 1895, pp. 268, 269), manifestly in error when he states, "Barth's suggestion has reference to the effect on host plant only." The subject of Barth's paper is, "Some new observations on the downy mildew (*Blattfallkrankheit*) of the vine." The first paragraph reads as follows: "An application of Bordeaux mixture on grapes at the proper time proved ineffectual (*unwirksam*) As the cause for this failure the author assigned the fact that instead of using equal amounts of blue vitriol and lime in the preparation of his mixture, he employed twice as much lime as copper. Consequently, the plant sap could not dissolve the copper." It thus seems that Barth's view coincided exactly with that recently expressed by Clark, namely, that the leaf is concerned in the solution of the copper and that this solution is the effective agent in the destruction of the fungus Whether Barth extended the same explanation so as to cover the action of the copper hydroxide on the fungus itself does not appear from the reference just quoted. At any rate, Rumm first showed the fact in the case of Spirogyra.

§Garden and Forest, vol. 7, 1894, p. 457.

‖See, however, Galloway & Woods, Proc. Am. Assoc. Adv. Sci., 1895, p. 53.

¶Ill. Exp. Sta. Bul. 68, 1902, pp. 157–188. For other literature, see bibliography on p. 101.

The writer has already given the results (p. 30) of some experiments which tend to show that copper hydrate is the most persistent and durable compound that exists in the Bordeaux mixture applied to leaves and left exposed to weather. It is to be remembered, however, that the methods there used do not correspond exactly to the conditions which hold when mixtures are sprayed on. Drops such as were there used dry up to somewhat thicker layers than if a thin stratum were deposited, as is the case when the substances are applied as a spray. Thick, isolated layers probably disintegrate more rapidly than thin ones. While the disintegration and removal of the different constituents of the Bordeaux mixture by the mechanical or solvent action of water when sprayed on foliage under normal conditions have a very important practical bearing in estimating the value of the mixture as a fungicide, and also evident importance in reference to the physiological action of the copper, etc., on the host plant, the matter here under consideration is first *the influence exerted by the simple presence of rain or dew on the leaves.*

Attention has already been called to the injurious local action of Cu $(OH)_2$ and $Cu_3O_2(OH_2)$ when applied as spots on apple and grape leaves during the summer of 1901 (p. 30). The weather record during the period between the application of these mixtures and the observation of this injury is interesting. In the interval between July 24 and August 17, during which time injury to grape leaves was observed, there was a rainfall of 8.46 inches (= 21.49 cm). The normal rainfall at Knoxville for this period, calculated from the averages of 30 years, is 3.33 inches (=8.46 cm), thus showing the rainfall to have been nearly three times the average. The period when similar injury was noted from the same applications to apple leaves was from August 24 to October 14 of the same year. During this period there was a rainfall of 6.66 inches (=16.9 cm), whereas the average precipitation for the time is 4.63 inches (=11.7 cm). No comparative experiments were made to determine whether rain and dew influence the injury of copper to grape and apple leaves, but from these considerations, the results of Lodeman and others above cited, and especially the striking results of experiments on peach foliage described below, there can be no doubt that the same results would have been obtained with grape or apple leaves, although for manifest reasons the phenomenon would be far less accentuated than is the case with the peach.

During several seasons of experimental spraying of peach trees with the Bordeaux mixture the writer has often been struck with the capricious behavior of the foliage thus sprayed. Sometimes no extreme injury would result even after several weeks, while in other cases very marked injury was shown in less than one week. The experiments here described prove conclusively that meteoric water is one of the most important, if not the most important, factor in determining the injury of Bordeaux mixture, etc., to peach foliage under normal conditions.

A number of different experiments were made showing definitely the fact just mentioned, but only two will be described in detail. The first test was made with potted peach seedlings of the former spring's germination. These experimental plants were similar to all the others referred to on former pages. In every case virgin soil was used as free as possible from

leaf mold, all from the same plot only a few yards square. The seedlings were taken from nursery when 4 or 5 inches high and set in pots, then put into greenhouse where they grew for several weeks. All the water cultures referred to in this bulletin were placed on trucks, being kept in the greenhouse under shelter at night and during showers, and rolled out under a wire enclosure during fair weather in the daytime. The wire covering this enclosure above and around was square-mesh galvanized poultry netting, the meshes being one-half inch square. This covering necessarily had some effect in protecting plants from dew, and this fact must be taken into consideration in this experiment.

On June 7 four similar seedling peaches in pots were thoroughly sprayed with Bordeaux mixture, two of the plants being set inside protected from rain and dew, the other two placed outside under the above mentioned wire enclosure and hence exposed to rain and dew. These plants were carefully observed from day to day. On June 15, eight days after spraying, unmistakable symptoms of copper poisoning were manifest on both the plants outside. By June 22 a number of leaves had dropped from these exposed plants. They were all left undisturbed until July 4, nearly four weeks after spraying, when they were photographed. Fig. 3, plate II, shows their appearance on this date, but represents merely the loss of leaves. Inspection of the plants showed every sprayed leaf remaining on plants outside to have suffered serious injury. Only four of these sprayed leaves were left on one, while two remained on the other; most of the leaves shown in the cut having grown out after the mixture was applied. In the case of the plants protected from rain and dew no injury whatever was apparent.

While this experiment appears at first glance conclusive, it is nevertheless conceivable that the somewhat modified effect of the sunlight on passing through the glass or the slight difference in temperature or moisture of the atmosphere or some other difference in conditions within and without the greenhouse may have determined this strikingly different result Therefore, an experiment was planned to test the matter directly.

Two potted seedling peaches similar to those above mentioned were sprayed with copper hydroxide on July 22, 1901. These plants were placed together on the ground in an open space exposed to direct sunlight, one being kept moist with distilled water, which was sprayed on at intervals of a few minutes during the greater part of the day, the other being kept dry. Both plants were kept under shelter at night. At the same time one twig each on four trees growing outdoors received the following treatment: One set sprayed with $Cu(OH)_2$ kept dry (except exposure to dew at night), another set with same preliminary treatment kept moist as above explained during the day, and a similar set of twigs sprayed with $Cu_8O_2(OH)_2$ with similar treatment.

On July 25, three days after application of $Cu(OH)_2$, the potted plant kept wet showed unmistakable evidences of copper poisoning by the appearance of red spots, which were especially numerous near the margins of the leaves. This injury rapidly increased until July 28, when the spraying with water was discontinued. At this time no injury whatever (except on youngest leaves, as usual) was visible on the potted plant that had been kept dry. At this date both plants were put under the above mentioned

wire enclosure and left there until August 2. 12 days after application of $Cu(OH)_2$ and six days after application of distilled water to one of the plants had been discontinued. During these six days four showers had fallen as follows: July 28, 0.01 inch (= 0.25 mm) in afternoon; July 29, 0.01 inch in morning; July 30, a trace of rain in late afternoon; July 31, a trace during night. Clear weather prevailed during the other days. The plant that had been sprayed with distilled water did not show much additional injury after cessation of spraying, but the reddened areas had begun to slough out, as usual, before August 2. On this date, red spots having just begun to make their apearance on the plant that had been kept dry, showing evident effects of six days exposure to dew and rain, the leaves from each were carefully removed and put in regular order in a flower press to dry. The contrast between the leaves kept wet and those kept dry was very striking; the former showing large reddened areas with a number of shot-holes, the latter, no shot-holes and only a few red spots. An effort to show the red spots by photographic process however proved futile, the copper hydroxide spots obscuring them. Plate III shows in upper row the leaves from plant kept wet, in lower row those from plant kept dry. The blotches faintly visible over surface of all leaves are copper stains, and do not indicate any injury. This plate merely shows the shot holes and browned areas, which were still manifest after extraction of chlorophyll with alcohol. It will be observed that the leaves kept dry show practically no injury except that ordinarily occurring on the young leaves at growing end of shoot. The leaves at base had begun to wither before being sprayed.

Although not quite so marked as in the case of potted plants, the effect of water in increasing the injury on the twigs of trees sprayed with $Cu(OH)_2$ and $Cu_xO_y(OH)_z$ was unmistakable. On the twigs sprayed with water the injury was quite evident in three days, while seven days had passed before it began to manifest itself on the check twigs kept dry, at which time the contrast was quite striking at a mere glance. In passing, it may be mentioned that a check set of shoots which had received no application of $Cu(OH)_2$ was sprayed with distilled water along with the others, in order to determine whether water standing almost constantly on the leaves in the hot sunshine could of itself produce injurious effects. After six days of such treatment no injury was observed.

In addition to the evidence thus far presented that moisture on leaves is a necessary condition for the injurious effects of copper, plate VIII shows in a very striking manner an exemplification of the same agency under normal orchard conditions. On the reader's left in that figure, where the Bordeaux mixture had not been followed with a spray of lime, the *outstanding, exposed shoots lost their leaves first.* The few leaves still remaining on the distal ends of these branches have partly grown out since the spraying and partly become rid of the areas of greatest injury by the normal process of exfoliation. All four trees treated similarly showed the same general appearance. The leaves nearer the center of the crown on the side not protected by a subsequent spraying of lime gradually dropped off until towards the close of the season only those sprayed with lime remained, and the crown was almost defoliated on the left side, even a little past

the center. The explanation of this phenomenon is very simple in the light of the foregoing experimental results. The central part of the crown of a tree in full foliage is well protected from deposition of dew and also to a greater or less extent from dew or rain dropping from above. It will appear from results stated below that sunshine also plays an important part in this injury and must likewise be taken into consideration here. The leaves on the outside of the crown are first exposed to the action of both water and sunlight and are thus the first to succumb to the fatal effects of the copper; then the next underlying leaves perish and fall in turn, until finally the tree is almost entirely defoliated. An impediment to this progressive injury is offered, however, by the rapidly growing buds at the ends of the branches, and may to a certain extent check its advance.

With the knowledge thus gained as to the necessity of the presence of water for the production of toxic effects of copper hydroxide on peach leaves, it is easy to understand the experiments made to determine the difference in action of copper applied on lower and on upper surfaces of the leaf. It was shown on p. 32 that in some cases copper hydroxide applied to lower surface of peach leaves produces no injury, in others its injurious action is far more rapid than when applied to the upper surface. In many cases where several of these spots were applied to the same leaf at the same time, injury appeared on one spot underneath far exceeding that caused during the same period on upper side, while other spots underneath entirely similar showed no injury whatever. The inevitable conclusion to be drawn from this is that in the presence of water the injury of copper hydrate to peach leaves is far greater when the copper is applied to the lower surface than when it is applied to the upper surface. When rain falls as a gentle shower, the lower surfaces of the leaves are scarcely wet; if it falls in large drops the mere impact of the drops on the leaf surfaces scatters spray in every direction, some accumulating on lower surfaces; if a gentle mist floats through the air, or if rain is blown about by gusts of wind, water again accumulates, some reaching copper spots on the lower surfaces and accelerating their injury to the leaves. This factor can, however, little affect the problem under normal orchard conditions, for neither water nor spraying mixtures adhere very readily to the lower surface of the peach leaf.

In the heading to this chapter sunlight is mentioned as a factor for discussion. No definite experiments were made to determine the isolated action of light, heat, air currents, etc., but potted plants were merely sprayed, some being left outside exposed to direct sunlight, others left indoors exposed merely to diffused daylight. Simultaneously with the above experiment on sprayed potted plants to determine the effect of the presence of distilled water on leaves in sunlight, an exactly parallel experiment was made on a pair of plants in diffused daylight and another pair in absolute darkness. Two plants in diffused daylight were put in an upper room on the north side of a building, and were situated about three meters from three large open windows on the north side, being thus exposed during the day to diffused daylight, but never to direct sunlight. Unfortunately no observations of temperature were made, but the plants in this room were necessarily

exposed to an atmosphere several degrees cooler than that outside, to say nothing of the absorption of heat rays by the leaves in the sunlight. Moreover, the freer circulation of air on the outside, tending to accelerate transpiration, must be taken into consideration. For determining the effect of total absence of sunlight, two potted plants were put on the floor in a photographic dark-room, where no light reached them except very subdued light for a few moments when the doors were opened in order to spray one of them with distilled water. The result of this experiment is briefly stated as follows:

All plants sprayed with $Cu(OH)_2$ on July 22, 1901.

Leaves kept moist while exposed to direct sunlight: injury began in three days. (Plate III, upper row).

Leaves kept dry, exposed to sunlight: no injury. (Plate III, lower row).

Leaves protected from sunlight, sprayed with water for six days: entirely healthy after several weeks.

Leaves protected from sunlight, kept dry: no injury.

Leaves in dark room sprayed with water: began to drop off after two days in darkness, all except youngest off in four days.

Leaves in dark room, kept dry: exactly as foregoing.

It thus appears beyond question not only that moisture must be present on the leaf for the injurious action of the copper, but that direct sunlight is also a necessary condition of this injury. The effect of darkness is manifested by such a rapid loss of leaves, even in the absence of copper, (p. 72) that no conclusions can be definitely stated on this point, but there can be no doubt of the total absence of evil effects of copper in total darkness, since no injury occurs in subdued daylight in the presence of water.

An experiment of somewhat similar character was begun June 26, 1901, by spraying with Bordeaux mixture four peach plants growing in pots. Two of these plants were left outside exposed alike to sunlight and rain during the daytime and to dew at night; the other two were placed at the end of a long hall, where only very subdued daylight could reach them, but were placed outside with the other pots at night and during showers in the daytime. The characteristic copper injury appeared distinctly on the plants outdoors at the end of two weeks, but no injury was shown on the plants kept inside. Later observations could not be made on account of the injury resulting to the plants inside from lack of light.

From these experiments there can be no doubt that peach leaves exposed to moisture in the sunlight after being sprayed with copper hydroxide suffer greater injury than those remaining in the shade under like conditions. The last experiment is especially instructive *in that it shows that the presence of dew on the leaves during the night does not produce so much injury as alternating showers and sunshine during the day.*

The question naturally arises in this connection as to what effect mere hygroscopic moisture has on the injury produced by copper on peach leaves. This becomes the more important in any discussion of the method by which copper enters the leaf, taken up in the next chapter, where it is shown that the leaves formed in a moist atmosphere have a thinner and more easily permeable cuticle than those grown in a dry atmosphere.

The experiments made for the solution of this problem were with peach seedlings growing in pots. On top of the pot rested a shallow pan of galvanized iron, with an open tube soldered in the center. Through this tube the leaf-bearing shoot of the plant was passed, the pan being filled with water after adjusting on rim of flower pot. A tall tubulated bell jar was then placed with its base resting in the water contained in the pan, thus enclosing all the upper part of the plant. The tubulure of the bell jar was left open to furnish the necessary change of air. One test was made with wet and dry bulb thermometers, which showed a difference of only a fraction of a degree, wherefore, so far as this investigation is concerned, the atmosphere to which the leaves were exposed may be considered saturated. The effect on growth of plants under these jars was striking in many respects. The peach leaves appeared to the eye much thinner and were of a decidedly paler hue than in the case of plants grown outside. Apple plants formed leaves of a paler hue as compared with those grown outside, and in one case a stem formed adventitious roots two decimeters above the soil.

On September 25, 1900, two apple and two peach seedlings in pots were sprayed with double strength Bordeaux mixture (3½ per cent $CuSO_4$ — excess of $Ca(OH)_2$) and placed outside exposed to weather conditions. At the same time two apple and two peach seedlings growing in moist atmosphere for several months were sprayed with the same mixture and left in the moist chambers.* On October 15, 20 days later, no injury was discernible on apple leaves either inside or outside of moist chamber; the peach leaves in moist atmosphere had suffered no injury whatever, while those left outside were almost defoliated. The peach plants in moist chamber remained some days longer before winter conditions began to be manifested, but never showed any injury from the copper.

The experiment was repeated, with certain modifications, on March 14. On this date one peach plant that had been growing in moist chamber in greenhouse for several weeks was sprayed with normal Bordeaux mixture and another similar one with copper-dicupric hydroxide. May 1, after six weeks, no injury whatever could be detected on the plant sprayed with Bordeaux mixture. On the leaves sprayed with $Cu_8O_2(OH)_2$, however, slight injury began to manifest itself at the end of two weeks. This injury was confined almost exclusively to the marginal teeth and the immediately adjacent region. At the end of six weeks, however, many leaves covered with $Cu_8O_2(OH)_2$ still maintained a perfectly healthy appearance; some leaves had also turned yellow and dropped off; but since unsprayed leaves under these abnormal conditions survive a very long period only with difficulty, some doubt existed as to how much of this injury was really due to the copper. The experiment with Bordeaux mixture was repeated again later with the same result as in other cases.

Unfortunately, at the time these experiments were made the writer was not fully aware of the part played by rain and dew in the injury of basic copper to peach foliage, and liquid water was never present very

*These are some of the plants from which specimens were gathered for measurements given on page 57.

long at a time on any definite area of the leaves in moist chamber. Under these conditions the moisture never condenses on the leaves*, but accumulates abundantly on the walls of the bell jar. In many cases, however, the drops thus accumulating fell on the leaves where the copper lay and remained there for a day or two without producing any apparent local injury. In others the growing shoots caused the leaves to bend down into the water, but in all cases without appreciable results. It would be a simple matter to determine beyond question how much the immunity of sprayed peach leaves in moist atmosphere is due to the mere hygroscopic moisture and how much to protection from deposition of liquid water on the leaf surface.† The frequent accidental presence of water on the leaves without apparent injury seems to indicate that hygroscopic moisture has a tendency to prevent the injury.

From the evidence presented under this heading the following conclusions may be stated:

1 Bordeaux mixture and pure basic copper produce no injury to peach foliage if no liquid water accompanies them.

2 In a saturated atmosphere, where water is not deposited on the leaves, there results no injury from Bordeaux mixture, and but little from pure copper hydroxide; this injury is also probably less than in a dry atmosphere under like conditions, even if water be present.

3 When protected from direct sunlight even the presence of water does not call forth this injury to peach foliage; but whether its immunity under these conditions is due to less light, less heat, or absence of strong air currents, or to the combined action of all these circumstances does not appear from these investigations.

4 While no direct experiments were made to test this matter it is still very probable that all the above mentioned conditions hold true likewise of the foliage of the apple and the grape.

5 Applied to practical orchard conditions, these results mean that rain in the daytime and, probably, the dew first falling in the evening are preeminently the determining factors in the injury of copper fungicides to foliage, the mere presence of dew or rain water on the leaves in the dark influencing this action to only a very slight extent. The outermost leaves on a sprayed tree being most exposed to dew, rain, and sunshine are the first to suffer injury, those on the interior following in regular order the falling of the more exposed leaves toward the ends of the branches.

It is now in order to recapitulate the experimental results recorded in this chapter and to determine their general trend.

In the first place, it is impossible not to be convinced that the injury caused by soluble copper salts sprayed on peach leaves is identical in char-

*Boussingault, l. c., p. 393.

†The fact that the injury from pure $Cu(OH)_2$ in moist atmosphere is almost exclusively confined to the region immediately surrounding the marginal glands seems to indicate that this slight injury occurs purely as a result of the entrance of water by the normal process of osmosis, and not by imbibition. (See p. 96.) Doubtless also the solvent action of the leaf secretions or excretions is less intense under these conditions. (Cf. p. 93.) The limitation of injury by $Cu_8O_2(OH)_2$ almost exclusively to the marginal teeth would thus be explained not merely by the absorption of water from the saturated air by the gum present there, but also by the presence of thinner cell membranes over the glandular surfaces (p. 53).

acter with that produced by basic copper; for not only are they identical in their external manifestations, differing to some extent in degree, never in kind, but also *individuals showing the greater susceptibility to the one show it also to the other.* The latter consideration may be taken as conclusive evidence that the identity of reaction between the leaf on the one hand and the various copper compounds on the other, apparent to the eye, is not apparent merely, but real. From the fact of the greater injury caused by deliquescent copper salts than by those that do not absorb moisture from the air and thus form solutions, it follows very probably that a copper salt must be in solution on the leaf before it can cause injury to that leaf. There appears no plausible reason why the same thing should not apply to the action of basic copper. It has been shown that copper hydrate produces no injury to the foliage when it is kept dry, and that this injury is increased to a very marked extent when water is present in the daytime either as dew or rain under normal orchard conditions or as an artificially applied spray. Moreover, the presence of certain deliquescent salts causes a marked increase of the injury caused by copper hydrate to the foliage; whether this increased action is due simply to their deliquescence or to their solvent action on the copper is immaterial for the present argument. In other instances, as in the case of common salt, etc., the addition of a saline substance may render the copper soluble, probably by the formation of double salts.

There is abundant evidence that pure copper hydrate is itself somewhat soluble in water, and especially in meteoric water. The well known poisonous action of water distilled from copper vessels shows the solubility of this substance, not as a metal, but as an oxide, hydrate, or carbonate. True this latter phenomenon results at an elevated temperature, but the well known experiments of Nägeli* with copper coins, etc., show clearly that metallic copper may be oxidized and taken into solution in the presence of certain algae. Copeland and Kahlenberg† found that enough copper from the pure metal in distilled water passed into solution to exert evident injurious action on lupine radicles in 24 hours. The question may be raised, however, as to how much of this solvent action was due to secretions from the plant cells. This question, however, will be discussed fully in the concluding chapter, and the part played by lime, in addition to special experiments looking toward the solution of this question, will be taken up there.

The experiments of Boussingault, Wille and others show conclusively that soluble salts may be absorbed by leaves under certain conditions (p. 66). The striking fact that the apple is so much more sensitive to copper poisoning when the copper is supplied as a solution through the roots than the peach, while the foliage of the peach is far more susceptible to copper injury than that of the apple, offers abundant reason for a thorough discussion of the thickness and permeability of the cuticle of the leaves of the three plants here under special consideration; for the explanation most readily suggested as to the part played by soluble copper on leaves is that it enters the tissues. In order to gain access to the interior of the leaf a substance must pass a membrane, the cuticle, well known to be of very variable thickness and of different degrees of permeability.

*Quoted by Swingle, Bul. No. 9, Div. Veg. Phys. and Path., U. S. Dept. Agr., 1896, p. 28.

†Trans. Wis. Acad., vol. 12, part II, 1898, pp. 454-474.

CHAPTER III

Permeability of the Cuticle and Its Relation to the Action of Fungicides

Before entering upon the main point here under consideration, viz., the relation of the thickness of the cuticle to the susceptibility of the foliage to copper injury, there are some special features pertaining to the structure of the peach leaf which demand some consideration. Attention has already been called to the presence of a varnish over the leaves in their early stages of development. The writer has made no test of this substance, but it is very likely identical, at least in part, with the secretion referred to above (p. 51). There are present on the margin of the leaves, terminating their dentations, bladder-shaped glands, or rather "glandular surfaces," as they are termed by De Bary.* These bladder-shaped glands are especially well developed and functionally active on the margins of the stipules in unfolding buds. This substance on drying closely resembles gum arabic in appearance, and on examination in alcohol under the microscope swells rapidly after access of water and is therefore to be classed with the gums.† This gum accumulates in the sinuses of the foliar teeth, sometimes filling up these recesses almost completely (Fig. 1a, plate IV) and extending over the leaf surface for an appreciable distance, imparting to the area thus covered a glossy appearance quite similar to that of very young leaves.‡ This accumulated gum softens in wet weather and may be washed out by rains and again freshly secreted, at least by young leaves. Careful examination of peach leaves growing under all conditions very rarely failed to show its presence in greater or less quantity. Bits moistened and applied to litmus paper show an acid reaction in common with gum arabic, etc.

Only one examination was made to determine the thickness of the cuticle covering this glandular surface. A mature leaf was taken on August 14, at which time the cuticle of the glands was fully as thick as that of the adjoining leaf epidermis. This clearly indicates that these glands are functional only during the youth of the leaf. It is a well known fact that in their functional stage glands have a very delicate cuticular covering, the cuticle sometimes being ruptured and regenerated.§

There are also present on the petioles of peach leaves (of the type used in these investigations) at the base of the lamina foliar nectaries, saucer-shaped structures, which secrete minute drops of fluid plainly visible under

*Comparative Anatomy of the Phanerogams and Ferns, pp. 96, 98.

†Zimmermann—Humphrey, Botanical Microtechnique, 1893, pp. 155, 156.

‡The writer has consulted on this subject only the literature here cited, and merely states here the facts observed which seem to have a possible bearing on the subject under discussion.

§De Bary, l. c., p. 98. See also Reinke, Jahrb. wiss. Bot., Bd. 10, 1876, pp. 127–131.

a lens. Small bees may often be observed in numbers gathering this secretion, indicating its sugary character. It also gives a very distinct reaction with Fehling's solution.

From the experiment described on p. 43 it is very evident that neither of these secretions can play any important part in producing the injury of copper hydrate to the leaves. Gum arabic and the very gum in question here failed to increase appreciably the injury due to copper. Gum arabic was applied in sufficient quantity (0.5 per cent solution) to produce marked effect if it had any such tendency, and although after carefully removing the peach gum in question from a large number of leaves not enough was obtained to make a strong solution, it was yet present in far greater quantity on the leaves sprayed with it than it can be under normal conditions. Yet, the peculiar location of this gum being such as to allow of its being removed with difficulty and its property in common with other gums of absorbing and retaining water for a time, make it probable that it may contribute, at least in part, to the greater injury produced at the margin of the leaf by copper. That it has no appreciable solvent action on basic copper the writer proved by placing a drop of water containing a quantity of this gum and some of the precipitated particles of copper-dicupric hydroxide on a slide and examining under the microscope. The dark brown color of this copper compound makes it distinctly visible, and although remaining several days in the moist chamber in contact with the gum these copper particles never showed any evidence of having gone into solution. The power of gums to retain moisture doubtless plays an important part in the injury produced by copper on peach leaves growing in a saturated atmosphere. Here, it will be remembered, the injury caused by pure basic copper is confined almost exclusively to the marginal teeth, which by the healing process discussed in Chapter IV are very neatly and uniformly sloughed out under these conditions. The question arises here whether this greater marginal injury is due to the greater permeability of the cuticle over the glands or to the mere presence of the secretion there. It will be shown on p. 59 that sulphur dioxide (or sulphur vapor) also produces greater injury to the margin, and for this reason it appears somewhat more probable, especially in view of some facts brought out later in this chapter, that this increased marginal injury is due simply to the *greater permeability of the cuticle over the glandular surface of the teeth.** It is especially to be remembered that lime prevents entirely the partial injury occurring to foliage in a moist atmosphere. While grape sugar was found (p. 44) to increase slightly the injurious action of copper hydrate on the leaves, the minute quantity secreted by the basal glands cannot play any appreciable part in the production of this injury. Moreover, if this secretion were capable of producing any marked results in this direction, leaves sprayed with copper would show appreciably greater injury in the basal region, a condition which has not been observed.

While the marginal glands especially must play an important part as affording the conditions for increased injury to peach foliage by copper, it must be remembered that they cannot be held responsible for all the injury

*Another possible explanation is that the protoplasm of the glandular cells is more susceptible to copper poisoning.

thus produced under normal conditions. It has already been shown that this injurious action of basic copper is for the most part local in its character, and that a spot of copper hydrate as far removed as possible from the margin of the leaf produces a marked local injury. For this reason a thorough investigation of the cuticle of the three kinds of leaves used in this comparative study is necessitated.

The first point to be considered is simply the histological difference manifest among the leaves of the three plants here under consideration. It has been long known that the cuticle of plants inhabiting arid regions or whose habitat in any region exposes them to external conditions having a tendency favoring rapid transpiration or reducing absorption of water by the roots, is in a general way much thicker than that of plants growing under the opposite conditions. At the same time many other conditions, such as the presence or absence of a hairy covering on the leaves, habit of growth exposing greater or less leaf surface to air currents, relative number and distribution of stomata, reciprocal relations between specific transpiration of shoots and specific power of absorption by roots, and perhaps many other factors, all combine in a more or less obscure way to produce great differences in thickness of cuticle among species growing under almost identical conditions in the same region. Thus it is that one may be led to suspect considerable differences in this particular among even closely related species and varieties, and from these physiological considerations one is led, even independently of the previously discussed experiments, to suspect some such structural difference between the apple and the peach leaf, for example, which may account for the fact that the former is only very rarely injured by the Bordeaux mixture, while the latter is almost without exception seriously injured. Bailey* examined sections of peach leaves microscopically, and observed that they have "a very delicate structure, the epidermis being remarkably narrow, with thin-walled cells," and accounted for their peculiar susceptibility to London purple by supposing that "the poison quickly permeates the tissue." He, however, made no systematic investigation of the question, and merely accounted thus for the severe action of arsenic already in solution before being applied to the leaves. Müller† accounted for the different degrees of susceptibility of leaves to spraying mixtures on the ground of different degrees of cutinization, and called attention to the necessity for investigations looking toward the determination of the specific susceptibility of the protoplasm of different fruit trees to copper poisoning—exactly the line of investigation in which the writer was engaged before Müller's publication.

In the investigations here recorded little attention was given to varietal differences, because in the first place any differences of structure noted must be common to all varieties of the peach, for, as compared with the apple and grape leaves, they all produce foliage far more susceptible to injury by fungicides than the latter two species. In fact, as a broad general statement (in this locality) it may be said that the leaves of all varieties of the peach are very much injured by copper fungicides, and that no apple and grape leaves of any variety suffer any injury. This, of course, does

*Cornell Exp. Sta. Bul 18, 1890, p. 35.

†Hollrung's Jahresbericht, 1899, p. 118.

not exclude varying individual and varietal degrees of susceptibility of the peach foliage, which has already been mentioned and discussed.

In addition to thickness of cuticle perhaps the thickness of the cellulose portion of the outer wall may be thought of as a factor in the phenomenon under discussion, but this evidently need not be taken into consideration, although measurements of it were made. The well known fact* that solutions of poisons pass through several cellulose walls of a multicellular hair whose outer walls are cuticularized before they can pass one cuticularized wall, clearly substantiates this statement.

In addition to these general physiological considerations may be mentioned a special point which suggested a possible explanation for the injury of fungicides to peach foliage. It is said that Bordeaux mixture does not injure peach leaves in California.† Kohl‡ found the cuticle to be appreciably thinner when the plant is grown in a moist atmosphere. Lothelier§ obtained the same result, and found that the cuticle sometimes even entirely disappears when plants are grown in saturated air. Since, therefore, the cuticle of the same species will be thicker or thinner according as it is exposed during the development of the leaf to a drier or moister atmosphere, the region above referred to offers the conditions for the development of a thick cuticle. At the same time it became desirable to know whether the peach leaf would show these variations in thickness of cuticle due to different moisture content of the atmosphere, hence the experiments in saturated atmosphere referred to in the former chapter. For the sake of comparison, apple leaves were likewise produced under similar conditions, and as will be shown later gave some very striking results of a confirmatory character. In all the experiments from which these measurements were made chloriodide of zinc was used as a stain. Microtome sections one and two micromillimeters thick were made from material embedded by the usual process in paraffine. Sections were invariably taken from the median region of all the leaves, care being taken to avoid the larger veins. Measurements were made by means of Zeiss' *Schraubenmikrometer* with Zeiss' 3 mm immersion objective. In this investigation no account was taken of the differences between the cuticle proper and the cuticularized layers. Evidently for the purposes of this investigation no such differentiation is necessary. Some notes are given, however, which indicate some of these points, and Fig. 2, plate IV, shows clearly these two regions of the outer epidermal wall of the apple leaf clearly differentiated. The measurements include the sum of the two. In the various measurements a cell with an average cuticle was always chosen from each section, so that the actual number of measurements might the more accurately represent the average. In cases where there was much difference in this regard measurements representing each extreme were made. The specimens were all gathered (except where otherwise stated) sufficiently late in the season to show total thickness of mature cuticle.

*Pfeffer, Pflanzenphysiologie, Bd. I, 1897, p. 101.

†Sturgis (l. c., p. 221) records a statement to this effect by the Yearbook of the U. S. Department of Agriculture for 1897, but the writer failed to find it after diligent search.

‡Die Transpiration der Pflanzen, 1886, p. 94.

§Rév. gén. de Bot., t. 5, 1893, p. 521.

In every case two representative sections were chosen for measurement from each individual leaf. The following table gives a summary of these measurements:

TABLE I Averages of Measurements of Outer Epidermal Walls of Leaves in Micromillimeters

		Upper Surface			Lower Surface		
		Entire Outer Wall	Cellulose Part	Cuticle	Entire Outer Wall	Cellulose Part	Cuticle
Apple from Knoxville orchard	5 leaves from 2 trees	3.76	1.99	1.77	2.06	1.22	0.84
Apple from pot in greenhouse	2 leaves from 2 plants	3.69	2.82	0.87	2.86	2.19	0.67
Apple from moist chamber	3 leaves from 3 plants	3.97	3.18	0.80	2.64	2.08	0.55
Peach from orchard, California	4 leaves from 4 trees	3.83	2.62	1.21	3.11	1.78	1.31
Peach from Knoxville orchard	4 leaves from 4 trees	3.17	2.03	1.17	2.69	1.53	1.17
Peach from pot in greenhouse	6 leaves from several plants	3.39	2.53	0.86	2.75	2.00	0.75
Peach from moist chamber	7 leaves from several plants	2.67	2.07	0.60	2.11	1.61	0.50
Peach from orchard, leaves young	5 leaves from several trees	3.01	2.09	0.92	3.04	1.86	1.18
Grape from vineyard	4 leaves from 4 plants	2.10	1.25	0.89	1.86	1.08	0.78

It would be transcending the bounds of this paper to discuss fully the results of these measurements from a physiological point of view, and the matter will be dismissed with a very brief reference to a few of the observations made.

In the first place, it may be assumed with almost absolute certainty that the thickness and the permeability of the cuticle of the same species growing under different conditions are not exactly proportional; and with far less probability can this proportionality exist in the case of the cuticle of different species under the same conditions. As was stated above, chloriodide of zinc was used to differentiate the cuticle for all measurements made. It was an easy matter to see that the cuticle of the apple leaf, even though the sections were of the same thickness, was stained far more intensely than that of the peach growing under like conditions. The cuticle of the grape was so poorly differentiated that measurements were made with difficulty The same striking difference in intensity of staining was evident between the cuticle of peach leaves from trees outside and that of leaves from moist chamber. In making photomicrographs it was found to be an easy matter to get actinic contrast sufficient to show the cuticle of peach leaves from trees by double staining sections with Sudan III* and hæmatoxylin; but in sections from leaves grown in moist chamber the cuticle showed such faint coloration from the former stain that it was scarcely distinguishable on the photographic plate.

*Buscalioni, Reprint from Malpighia, vol. 12, pp. 7-9

Careful inspection of table I will show a number of interesting points as to the effect of exposure, etc., on the development of the cuticle. In this connection it is well to call attention to the fact that the cuticularization of the upper epidermis is the only feature of histological character that can be of special value in this investigation. The presence of stomata on the lower surface, as well as the very striking cuticularization of the inner epidermal walls, here render any calculation as to permeability almost worthless. But some very instructive lessons may be drawn in a general way from a comparative study of the upper and the lower cuticle.

In the case of both apple and grape leaves there is a considerable difference in thickness of cuticle between the upper and lower epidermis. In the apple leaf from orchard the cuticle of upper epidermis is to that of lower as 1:0.47, over twice as thick. In moist chamber this proportion is as 1:0.69, a proportional increase for the lower cuticle of 47 per cent. The upper cuticle of grape leaf under normal conditions is to the lower as 1:0.87. In the peach leaf from orchard at Knoxville the upper cuticle is equal in thickness to the lower, from California orchard the lower is the thicker, the proportion being 1:1.08. The manifest explanation for this difference in proportion between the upper and lower leaf cuticle in these three species is the presence of a hairy covering in the case of both apple and grape on the lower leaf surface, which covering is entirely lacking in the peach leaf. Moreover, the conduplicate vernation of peach leaves, persisting to a certain extent after the leaf emerges from the bud, is of such a character as to expose the lower surface more to air currents, etc., and hence another demand for thicker cuticle to protect against excessive transpiration. This correlation between exposure and thickness of cuticle is strikingly shown by the measurements above given of cuticular thickness of peach leaves growing under various conditions. In the first place, the upper cuticle of leaves from California measured 1.21 micra, from orchard Knoxville 1.17 micra, from pot in greenhouse 0.86 micra, from saturated atmosphere 0.60 micra, thus showing an unmistakable gradation in thinness of cuticle as the leaves grew in a gradually moister atmosphere. The same gradation is shown in the apple leaf. From orchard at Knoxville the upper cuticle averaged 1.77 micra, from pot in greenhouse 0.87 micra, in moist chamber 0.80 micra. A comparative study of the ratio between the thickness of the upper and lower cuticle of the peach leaf under these varying conditions is most interesting. In the California leaves the ratio of thickness of upper to lower cuticle is 1:1.08; leaves from Knoxville, 1:1; from pot in greenhouse, 1:0.87; from moist chamber, 1:0.83. As above stated, the ratio between the upper and lower cuticle of apple leaf from orchard is 1:0.47, while the same ratio of leaf from moist chamber is 1:0.69. These apparent differences in behavior of apple and peach foliage afford an interesting example of the opposing tendencies on the part of the organism in one case to adapt itself to its environment, in the other to follow its inherited predisposition.

Finally, the most important results obtained by these measurements are the relations between the cuticle of the leaves of the three species of plants grown under normal conditions. As above stated the cuticle of the lower epidermis can show nothing very definite for our purpose. The upper

cuticle of the leaves grown in this latitude under normal conditions gave the following average measurements: grape 0.89 micra, peach 1.17 micra, apple 1.77 micra. Taking the thickness of the grape leaf cuticle as a unit, the proportion stands: grape 1, peach 1.31, apple 1.99. A number of photomicrographs were taken of the cuticle of peach and apple leaves, of which only one, that of the apple, is shown in Fig. 2, plate IV.

As above mentioned, the mere thickness of the cuticle does not accurately indicate its permeability. Some experiments were undertaken with the view of determining the relative permeability of the leaves of the three species here investigated. While this investigation is still in its incipiency, yet some results of a preliminary character have been obtained which agree so thoroughly with the other results set forth in this paper and are so concordant with the general physiological principles involved, that a brief description of them is given.

In the first place, an accident occurred in the course of these experiments which proved to be very instructive in more than one respect. Nearly all the experimental plants, including water cultures and pot cultures of apple and peach seedlings, both within and without moist chambers, were seriously attacked by red spiders. On August 17, 1900, the trucks bearing water cultures were rolled inside the house, the bell jars over apples and peaches in moist chambers were removed, and after shutting all windows and doors, sulphur was vaporized according to the usual method for destroying these pests. The experiment was left for a few minutes in charge of a laborer, who allowed the sulphur to take fire for a very short time. The plants were thus exposed for about a half-hour to sulphur vapor and for a much shorter time to sulphur dioxide gas. It is thus impossible to state definitely which of the two substances was responsible for the injury about to be described, but for the purposes of this investigation this is an unimportant matter. On the next morning after this fumigation, almost every apple and peach leaf grown in the saturated atmosphere was withered and dead. On the apples growing in pots and culture jars outside of moist chambers not a single leaf was injured. On the peach plants outside of moist chambers a still more instructive phenomenon was visible. Only the very young leaves near the terminal buds of the growing shoots were here injured. The buds themselves were not killed, but continued to grow. Near the close of the growing season on almost every potted peach the location of the injury caused by the sulphur was plainly distinguishable by the absence of leaves from several nodes. The peaches in water culture suffered practically no injury, probably because the plants not being very vigorous, the apical leaves were older and more thoroughly cuticularized. But the most instructive point observed in this accidental experiment was the behavior of some of the leaves on the border line between youth and maturity. Here no injury was observable until four days after the accident, when several of these leaves on potted plants and one in a water culture showed the characteristic exfoliation of a marginal strip about two millimeters wide nearly all around the leaf. (See plate VI, Fig. 1). There can be no doubt that the relative thickness of cuticle already referred to is responsible, at least in part, if not altogether, for the occurrence of injury in some leaves and of immunity in others. The

apple leaves in moist atmosphere have been shown to have a thinner cuticle than those in pots—the former were the only ones to suffer from sulphur poisoning. Young peach leaves have a thinner cuticle than mature ones—the young ones died. Mature peach leaves in moist chamber have a thinner cuticle than mature leaves in a drier atmosphere—only those in moist chamber perished from copper. The peach leaf itself has definite areas along the margin (glandular surfaces) which have a thinner cuticle than the rest of the lamina. It is plain that some leaves may be found with just such difference between development of cuticle over glands and cuticle over general leaf surface as to allow the entrance of sufficient gaseous poison to injure the marginal region without injuring the rest of the leaf. Fig. 1, plate VI, shows one of several such cases. In this figure is also seen in the median region of the leaf evidence of the same local points susceptible to injury by sulphur as was above (p. 33) seen in the case of copper hydrate.

Attention may be called also in this connection to a similar effect of lithium carbonate already referred to (p. 41). Among the different substances sprayed on peach leaves in this experiment, the last mentioned salt was applied as a 0.5 per cent solution. It proved very destructive to the foliage, and always showed injury in the marginal region sooner than in the median region of the leaf. Whatever explanation may be offered for this peculiar local action of one poison must apply also to the action of them all. The relative thickness of the cuticle has such manifest relation to this local action as to require no further discussion.

The method of Boussingault* for determining the entrance of soluble salts into leaves is readily suggested as at once the simplest and most natural means for determining the relative permeability of different leaves for salts in solution. The time required for the disappearance of a salt kept wet on a leaf ought to be at least an approximately accurate measure of the permeability of the cuticle of this leaf for the salt in question. It was the intention of the writer to attempt an investigation according to this method during the summer of 1901, but he was unable to do so.

The permeability of the upper surface of a leaf to distilled water may be taken, especially in the light of certain citations to be given presently, as at least an approximation to the permeability of the cuticle for salts in solution. As a rule, substances which can enter the cytoplasm are able also slowly to traverse the cuticle.† There seems to be no very accurate method known for estimating the relative permeability of such membranes as leaf cuticle, cork lamellae, etc. The question has been discussed by various writers, among whom may be especially mentioned Hofmeister, Zacharias, and Eder. Hofmeister found‡ no passage of water through young potato peel to concentrated gum or sugar solution, and no filtration of water under pressure of 300 mm of mercury. The same corky layer was tested by Zacharias§ with similar results under a pressure of 430 mm mercury. Eder‖ estimated roughly the permeability of cuticularized and

*L. c., p. 385.

†Pfeffer, Pflanzenphysiologie, Bd. I, 1897, p. 76.

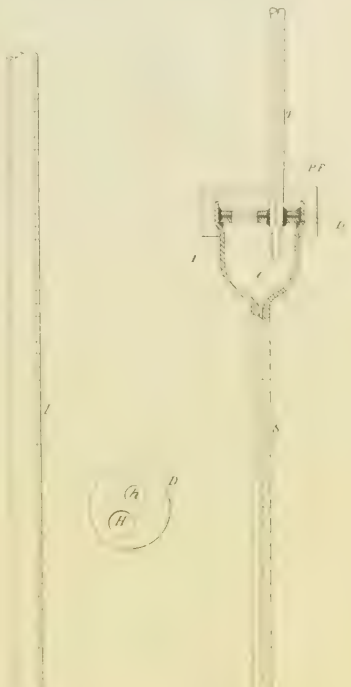
‡Die Lehre von der Pflanzenzelle, 1867, pp. 238, 239.

§Bot. Zeitung, Bd. 37, 1879, p. 633.

‖Sitzungsb. Akad. Wien, Bd. 72, 1875, p. 258.

suberized membranes by the time required for passage of water sufficient to moisten a dry, soluble salt on the other side, or if diosmosis was sufficiently rapid, by its rate as indicated by amount of water passing to the salt solution. Not sufficient difference of pressure was allowed on the two sides of the delicate membrane to rupture it.

The great objection to all such methods of estimating permeability of the cuticle is that the experimental procedure necessarily involves the death of the cells, and nothing very definite is learned as to the permeability of the membrances in question under normal conditions. It will presently be shown on good authority that even during life the epidermis of the leaf is subject to considerable fluctuations in permeability in response to certain changes in external conditions; and if the membrane undergoes these changes during life, for a greater reason this might occur on passing from the living to the dead condition. The fact pointed out by Wille* that old leaves in which destructive processes have already set in take up salts more readily than those in their prime, is sufficient evidence for such an assumption. In the investigation of another problem where this variant permeability does not perhaps come so much into play, the writer designed an apparatus for testing the permeability of delicate membranes, with which only one comparative test was made for the solution of the problem here proposed. This experiment cannot be considered conclusive, but taken in connection with all the other evidence here set forth it must have considerable weight of a confirmatory character.



The accompanying cut shows a diagram of the apparatus. It consists of five separate pieces, a long L shaped glass pressure tube, the lower part of which is shown at L; a short arm S communicating with L by stop-cock joint; two doubly perforated metal discs D; and a small thermometer, whose base is shown at T. The tube L is 9 mm bore and graduated in mm for a length of one m. The short portion below, bent at right angles, communicates as shown by dotted lines with capillary bore of S by means of stop-cock joint. S carries stop-cock fitting for short branch

*Cohn's Beiträge, Bd. 4, 1887, p. 319.

of *L* below, which communicates directly with the ¾ mm bore of middle portion of *S*. This middle portion of *S* communicates with cup *C* above. The cup *C*, as shown in section, is flared at the rim above. This thermometer tube of ¾ mm bore is graduated in mm beginning with its zero at same level as zero on long arm, this scale on the short arm extending for 150 mm. At *p* is a perforation through wall of female stop-cock connection on a direct line with capillary bore of *S*, thus permitting, after removal of *S* from *L* by slipping off at stop-cock connection, the introduction clear into the cup *C* of a minute capillary tube explained below. It is readily seen that communication between *L* and *S* may be cut off by turning *S* upward from plane of paper about the stop-cock joint as an axis, so as to bring *S* into a position approximately perpendicular to plane of *L*. The iron (some noncorrosive metal would be better) discs *D* are 2 mm thick, 30 mm in diameter, and contain two perforations, *H* 10 mm in diameter and *h* of sufficient size to admit loosely the bulb of thermometer *T*. The discs are slightly beveled on the perimeter, thus forming a shallow trench around edge when put face to face. The perforations *h* and *H* are so placed in the two discs that each coincides with its fellow when they are placed together. These discs fit loosely into the cup *C*, and are shown in radial section in place in cup at *D*.

In use, a circular disc somewhat larger than the perforation *H* is carefully cut from a leaf and cemented over *H* on one of the discs by means of a mixture of asphalt, beeswax, and resin. This cement is spread by means of a warm needle over the entire surface of the metal disc, care being taken to prevent its spreading over the leaf surface not in contact with the metal. Then the other disc is slightly warmed and carefully adjusted so as to make the corresponding perforations exactly coincide. Thus cemented together the discs carrying the piece of leaf are together cemented into the upper part of the cup *C*, as shown in section at *D*. In the experiment described below the lower surface of the leaf was placed inward. The thermometer *T* is next cemented in place as shown in cut, after which a plaster of Paris covering *PP* is placed over the whole as shown and allowed to set. Especial care is necessary to insure intimate contact between the freshly mixed plaster and the outer leaf surface. This is best secured by placing a few drops of water on the leaf surface, dropping in the powdered plaster, and carefully stirring for a moment with a camel's hair brush. This thin layer begins to harden after a few minutes, when the whole covering of plaster may be added and allowed to set. As soon as the plaster has sufficiently hardened, the arm *S*, which of course has been disconnected during the whole operation, is inverted and the female stop-cock joint filled with distilled water.

The water will of course not enter the cup through the narrow tube communicating with it. The whole process of cementing must be done when the apparatus is dry, and in order to compel the entrance of the water through the capillary bore a glass tube was drown out to a long, straight, exceedingly narrow point, and a rubber bulb connected with the larger end, thus constituting a sort of medicine dropper with an extremely long capillary point. After squeezing this bulb and inserting the capillary point through the aperture *p* into the cup *C*, and allowing the bulb to

expand, the air in C being sucked out, the water rushes by into the cup C. It thus requires only five to 10 minutes to fill the cup. By means of this capillary tube attached to rubber bulb a minute air bubble may be removed from any part of the cup after shaking. The thread of mercury may also thus be made to assume any desired position in the calibrated tube. By careful rotation of the stop-cock joint after pouring mercury into long arm L, communication between the two mercury columns may be had without presence of air or water bubbles.

After filling with water, the apparatus is set aside for the plaster to harden and dry, which requires usually 24 to 36 hours. After this has occurred the apparatus is carefully adjusted with both arms in a vertical position, and, the mercury column in calibrated tube S having been previously brought to zero on the scale S, mercury is poured into L until its level is brought to the desired height, and the temperature of the water in cup C is noted. The escape of water through the leaf surface at I is indicated by rise of mercury in calibrated tube S, and is noted from time to time. This filtration is very slow, as found by experiment, and this small quantity of water is absorbed by the bibulous plaster of Paris without causing it to soften. The leaf does not suffer rupture because all its minute surface inequalities are protected by the plaster above. On making subsequent readings, care must of course be taken to bring the temperature of the water in C to the same degree as that observed on first reading, which may be accomplished by holding warm or cold bodies against the outside of cup C. When pressure is first applied, a sudden rise of mercury in short capillary tube indicates either a leak or the slight adjustment of the discs, which, owing to the viscous character of cement used, may move upward slightly on being subjected to pressure. If mercury continues to rise rapidly a leak is indicated, and the experiment must be repeated. If the column moves up rapidly for a few minutes and then stops, the apparatus may be carefully disconnected, the mercury thread in S readjusted as at first, and then set up again.

It was found by experience, especially before the finally perfected form of the apparatus here figured was used, that a considerable proportion of the experiments failed by reason of imperfections in leaf surface or imperfect cementing. But the rule above given for determining the success or failure of the experiment proved reliable. A number of experiments were made extending over a period of about two months, which with additional experiments the author expects to make the subject of a future paper.

On the principle that the permeability of a membrane (to water) is represented by the rate of filtration through a unit surface of that membrane in a unit of time under unit pressure, this method of investigation above described was carried out. Pfeffer* found filtration under pressure through precipitation membranes to be proportional to pressure, other conditions remaining the same. It is to be remembered, however, that from the very nature of the membrane here under consideration, a number of experiments must be made before anything like mathematically accurate results can be hoped for. There are considerable differences in thickness of cuticle (to say nothing of degree of cuticularization) over even

*Osmotische Untersuchungen, 1877, pp. 108, 109.

small areas of the same leaf, and it is well known that the cuticle is more permeable over the veins of leaves than over the intervening surface.*

All measurements are in millimeters unless otherwise stated. The following indicates the method of computation:

R=reading of mercury level in L at beginning of experiment. (This changes so little in the course of an experiment that it may be left out of consideration).

r=reading of mercury level in S at beginning of experiment.

r'=same reading at close of experiment.

w=height of water column from r to leaf surface above.

w'=same from r' to leaf surface above.

c=capillary depression of mercury in calibrated tube of S.

p=average pressure on leaf surface during experiment, expressed in mm Hg.

a=area in square mm of leaf surface exposed.

a'=area in square mm of cross section of calibrated bore of S.

D=duration of experiment in hours.

F=average rate of filtration in cubic mm of water per square cm per hour during experiment.

P=permeability of leaf cuticle as expressed in average filtration of water through 1 square cm in 1 hour under pressure of 1 mm Hg.

$$P=\frac{F}{p}$$

$$F=\frac{100a'(r'-r)}{aD}$$

$$p=R-c-\frac{r+r'}{2}-\frac{w+w'}{27.2}$$

Where a set of tubes of same dimensions, with same leaf surface exposed, are set up at the same time and observations are made at the same time, a, a', and D become constants, when relative permeability is expressed by the following formula:

$$P\propto\frac{r'-r}{R-c-\frac{r+r'}{2}-\frac{w+w'}{27.2}}$$

The one experiment above referred to, made with a view to determining the relative permeability of the leaves of the three plants here investigated, was begun on August 23, 1900. In this experiment tubes of somewhat different pattern from that shown in cut were used, but they operated on exactly the same principle and were, so far as accuracy is concerned, just as efficient, except that the temperature could not be controlled so well, and varied about 0.1° C. This, however, cannot seriously affect the result. Late in the afternoon on the date mentioned a disc was cut from carefully selected leaves of grape, apple, and peach growing under normal conditions, and set up as above explained in similar tubes. By the morning of August 25 the plaster was thoroughly dry, and pressure was applied just before noon. At 1:07 p. m. an observation was made at 31.1° C. A number of observations were also made at different temperatures which

*Burgerstein, Jahresb. Leopoldst. Obergymn. Wien, 27, 1891, p. 22.

it is needless to record; the final one was made at 5:38 p. m., August 26, 28.5 hours later; temperature 31° C. In the apparatus here used a=78.54 qmm, a'=0.7854 qmm. The subjoined table gives the results of this experiment calculated by above formula:

	Av. Pressure in mm Hg. (p)	Elevation of Hg. in S. ($r'-r$)	Permeability (P). (Filtration of water through 1 qcm per hour, under pressure of 1 mm Hg)
Apple leaf	310.06	24	0.00271
Peach leaf	318.78	36.5	0.00401
Grape leaf	285.22	66	0.00810

A comparison of the permeability of the cuticle of these three leaves, as thus estimated, with the thickness of the cuticle affords a very interesting study. It has been generally assumed that the permeability of cuticle is, roughly speaking, inversely proportional to its thickness, at the same time also that the degree of cutinization must be taken into consideration. The following comparison is made on this basis:

	Apple	Peach	Grape
Relative permeability of cuticle (apple leaf as unit)	1	1.48	2.98
Relative thickness of cuticle (inversely stated)	1	1.31	1.99

This shows that while the cuticle of the apple leaf, for example, is only about twice as thick as that of the grape, *its permeability is about three times less.* In view of what has already been stated as to the degree of cutinization of the upper epidermis of the apple leaf as compared with that of the peach and the grape leaf (p. 57), as indicated by the intensity of coloration resulting from various reagents, the result here indicated receives a very plausible explanation. In fact, all the results here outlined as to the structure of the epidermis and its relation to climatic and atmospheric conditions, and its physical properties permitting the passage of water, agree in such a striking manner as to admit of no doubt as to their approximate accuracy and to demand no further discussion or explanation.

The statement was above made that under normal conditions the cuticle is subject to considerable variations in permeability. The writer has made no experiments in this direction, but there is abundant evidence to this effect in physiological literature. This naturally opens the question of the absorption of water by leaves, on which subject there is an abundant literature. Any effort at an investigation or discussion of all that has been written on this subject would clearly be out of place here.

Burgerstein* has published a thorough review of this literature up to 1891. He reached from the then accumulated literature, among others, the following conclusions:

"1 Foliage leaves are able to absorb liquid water through their upper surfaces."

"2 This absorption of water has been shown to occur in leaves where the epidermis is smooth and where it is provided with hairs; where it has and where it has not stomata; where the cuticle is thin or faintly cuticularized and where it is thick or strongly cuticularized; where the epidermis may be wet with water and where it is covered with wax."

*L. c.

"3 In general the plants in which this absorption of water by leaves has been shown reveal a wide range in organization, mode of life, and systematic position. Therefore, since no exception has been shown, it may be assumed that all plants have the power of absorbing water directly through the leaves."

"4 The amount of aqueous absorption depends on the anatomical structure and the relative water content of the leaves. It is furthermore clear that leaves which have suffered loss of water—up to a certain limit—can absorb more water than those approaching full turgor. . . ."

"5 Water can reach the interior of the leaf through the epidermal cells, through the hairs, and through the stomata."

"6 The lower epidermis of the leaf absorbs more actively than the upper—as investigations thus far show. As a rule three factors combine to cause this greater absorption by the lower side of the leaf: (a) the fainter cuticularization of the outer walls of the epidermis cells; (b) the greater abundance of hairs; (c) the greater number of stomata. . . "

There can be no doubt therefore that leaves take up water through the epidermis. It now remains to decide whether they may in like manner absorb salts or other substances in solution. This is also a well known fact.* Boussingault† found not only that leaves may thus take up water, but also at the same time will take up saline substances in solution. *This author found that relatively rapid absorption of substances in solution occurred under exactly the same circumstances where rapid absorption of water occurs.* He also found that a sparingly soluble salt, such as calcium sulphate, was more readily taken up than some more soluble ones.‡ Especially instructive also in this connection are the investigations of Wilson,§ who found that water exosmoses from cells of strongly cutinized leaves, such as Buxus, towards strong solutions of $Ca(NO_3)_2$, etc. In Wilson's experiments the leaves were completely turgid, and there was consequently an outward current of water through the cuticle in accordance with the normal process of osmosis. If, however, the leaves had been slightly wilted there can be little doubt that there would have been at first a movement not only of water but also of at least traces of the dissolved salt in the opposite direction. This would have been due not to endosmosis, but to imbibition.

Bailey‖ records an experiment with London purple on peach foliage which is especially interesting in this connection. He applied this insecticide to a tree on July 7, and "immediately thereafter the hose was turned

*Pfeffer, l. c., p. 143.

†L. c.

‡Reference has already been made to the inadequacy of Aderhold's (l. c., p. 255) experiments designed to show that calcium sulphate cannot be taken up by leaves in sufficient quantity to aid appreciably in the nutrition of the plant under normal conditions. Aderhold's experiments were made with water cultures, where all the tissues, including the leaves, must be constantly in a state of almost complete turgidity. This is scarcely true of plants under normal conditions. If he had allowed his water cultures to suffer alternate wilting and recovery with the presence of lime solutions on the surfaces of their leaves, his results might have been different. The fact above mentioned (p. 40), that water cultures show decidedly less injury to their foliage from copper sprays than pot cultures, affords striking evidence in this direction.

§Unters. bot. Inst. Tübingen, Bd. 1, 1881, pp. 3, 4.

‖Cornell Exp. Sta. Bul. 18, 1890, p. 33.

upon it and the foliage was given a thorough washing." Bailey had shown his mixture to contain soluble arsenic, and *in the short interval between spraying with the solution and immediate washing off* enough poison had been taken up to show "scorched spots" on the leaves within a week. Cook* had observed greater injury from same mixture if applied just before a shower. These authors do not state the exact time of day when their experiments were made, but their results are readily understood from the point of view here presented, and give very positive evidence of the correctness of the conclusions stated below.

There are two more investigations which deserve especial attention in this connection. F. Haberlandt† showed that leaves on cut off shoots wilt in less time if their surfaces had been previously wet than if they had been cut off while dry. This author found that vigorously transpiring leaves on a shoot have the power even to maintain their turgidity at the expense of the more succulent tissues toward the growing apex (flowers or developing leaf buds). Wiesner‡ extended the same observations to leaves still in communication with the roots under normal conditions. He concludes as follows:§ "The cause for the increase of transpiration and conductivity for water on the part of organs that have been wet is to be sought in a change of the walls of those cells that have come into direct contact with water. The membranes expand and thus, by enlargement of their water passages (*Wasserwege*), their capacity for resisting transpiration is diminished. When the transpiration current (*Leitungsfähigkeit*) is reduced (as in leaves removed from the shoot) there occurs a rapid drying out of the organs that have been wet."

From the experiments and discussion presented in this chapter the following conclusions may be drawn:

1 Grown under normal conditions at this latitude, the cuticle of the apple leaf (upper surface) is thicker than that of the peach, and that of the peach is thicker than that of the grape leaf, the proportions being respectively 1.99: 1.31: 1.

2 The cuticle of the apple leaf, and especially that of the peach leaf, show in common with other plants considerable variations in thickness, dependent on the medium in which the leaves are developed; exposure to a dry atmosphere, to strong air currents, in short, to all the atmospheric conditions that have a tendency to increase transpiration, results in an increased thickness of cuticle. The cuticle of a young peach leaf, especially on the upper surface, is considerably thinner than that of a mature leaf.

3 The permeability of the cuticle depends on its thickness and on the character of the various substances with which it is coated or impregnated. This permeability may be estimated by the rate of filtration of water under pressure or by the relative rapidity of action of gaseous or liquid poisons. The cuticle is more permeable over the veins than over the rest of the leaf surface. As measured by these several factors, singly or collectively—

*Mich. Exp. Sta. Bul. 53, 1889, p. 319.

†Wiss.-prakt. Unters. Geb. Pflanzenbaues, Bd. 2, 1877, pp. 130–137.

‡Sitzungsb. Akad. Wien, Bd. 66, 1882, pp. 209–265.

§L. c., p. 264

(a) The upper cuticle of the grape leaf is more permeable than that of the peach, which in its turn is more permeable than that of the apple.

(b) The cuticle of young peach leaves and peach leaves grown in a saturated atmosphere is more permeable than that of mature ones developed under normal conditions.

(c) The marginal glands present on peach leaves offer surfaces more permeable than the rest of the leaf.

4 In addition to specific differences in cuticular permeability of leaves and differences depending on habitat, etc., the permeability is subject to considerable variations in the same leaf, resulting in greater absorption of water when the leaf is in a wilted or semi-wilted condition and in a relatively greater loss of contained water after its surface has been wet.

5 Substances in solution in water placed on leaves are subject in a general way to the same conditions affecting their absorption by the leaf as influence the absorption of water by the leaf. This absorption may result from the ordinary process of endosmosis, but to a considerably greater extent from imbibition. Substances sparingly soluble are, other things being equal, taken up more readily than extremely soluble ones

CHAPTER IV

Wound-Healing of the Leaves and Leaf-Fall

Any comparative study of the relation of the three plants here considered to fungicides must include some consideration of the method of wound-healing and the conditions influencing the falling of the leaves. In fact, to the casual observer the most striking difference in the results of spraying peach trees as compared with similar treatment of the apple and the grape, is that after a few weeks the few leaves remaining on the former are riddled, while the latter two reveal neither loss of their leaves nor injury to them.

The writer has not made a thorough investigation of this subject, but has obtained a few results which may here be given in detail.

Attention has already been called to the local action of copper hydrate in producing injury to the leaves of the peach, apple, and grape. In the case of the peach, very rapid healing follows these local injuries, resulting not only in the separation of the dead from the living tissues by a layer of suberized cells, but in the eventual removal of this region from the leaf by mere shrinking and dropping out. This loss of any limited region by whatever cause is commonly known as the "shot-hole effect." The injured regions of the apple and grape leaves, for example, are not thus removed after callus formation, but dry up and turn brown, remaining connected with the living tissue until the whole leaf falls or this dried region is accidentally broken out by winds or other agencies. The peach leaf (in common with others of the genus Prunus) in comparison shows usually a neat and smooth delimitation of the missing injured region, not unlike in appearance the normal margin of the leaf. Blackman and Matthaei* have recently discussed this phenomenon in the case of Prunus laurocerasus. Duggar† found that this peculiar habit of exfoliation is possessed in common by a number of species of the genus, and there can be no doubt that the general method of healing in the peach leaf agrees with that found by Blackman and Matthaei in the species just mentioned. Fig. 2, plate V, represents the progress of wound-healing in the peach leaf under normal conditions in 10 days after injury. The injury was in this case produced by simply cutting through the margin of the leaf with a razor some distance toward the midrib. In the cross section through this wound photographed as shown in this figure, the collapsed cells to the right of the line *bb* died as a result of the injury. The cell walls in the region between the two dotted lines *aa* and *bb* are all suberized, as shown by lighter color resulting from stain used. The cell walls adjacent to suberized region are appreciably thickened. Very rapid cell division has

*Annals of Bot., vol. 13, 1901, pp. 533–546.

†Proc. 19th Ann. Mtg. Assoc. Prom. Agr. Sci., 1898, pp. 1–7 (separate).

occurred, even the epidermal cells sharing in the healing process. This photo-micrograph shows that under normal conditions the wound-healing process in the peach leaf agrees in all essential details with that observed by Blackman and Matthaei* for Prunus laurocerasus. But under the action of copper poison the peach leaf, as already pointed out by Duggar, exercises the same process of exfoliation as that described by the aforementioned authors for the species with which they experimented. Fig. 1, plate V, shows plainly the process repeated several times, as a result of local injury from copper-dicupric hydroxide. Duggar showed this peculiar stimulus, resulting in the so-called shot-hole effect, to be produced on a number of species of the genus Prunus by many poisons and also by a number of pathogenic fungi.† The latter author explained this response on the part of the leaf to poisons and to certain fungi on the ground of a similar reaction of the host to various poisons, the fungus "probably causing the death of the host cells by the excretion of certain deleterious ferments."

Blackman and Matthaei‡ found that this process of exfoliation is due to the formation within still living tissues of an absciss-layer, the middle lamella along a row of cells in a plane directly across the leaf being dissolved, while the cuticle of both surfaces is simply ruptured in the same plane, thus exfoliating the area concerned. These authors experimented with detached leaves in a moist atmosphere. Under normal conditions they were not able to produce the reaction by artificial injuries, but found formation of normal periderm of suberized cells similar to that shown in Fig. 2, plate V. These authors had evidently not seen Duggar's paper above cited, which was published three years before. They would here have found not only 'a description of the process of exfoliation which they had observed,' but also the real character of the stimulus which calls forth this reaction, as well as its teleological significance. As stated above, Duggar showed the same effect to be produced under normal conditions as a result of attacks of various fungi. The writer has already referred to the same phenomenon, which results in the exfoliation of the whole margin of the peach leaf (Fig. 1, plate VI) as a result of the stimulant action of sulphur vapor or sulphur dioxide, by reason of its ready access to tissues of leaf through the thin cuticle covering the marginal glands. The writer has frequently observed in leaves which have turned yellow and are just ready to drop as a result of the attacks of fungi or of local injury from copper sprays, that the region immediately surrounding these localized injuries has still a healthy green color in strong contrast to the yellow color of the rest of the leaf. This phenomenon is readily explained. The zone of occlusion marks the boundary line between the green and the faded region, the absciss-layer having been formed before the dissolution causing the yellowing of the leaf had set in. The substances resulting in the disorganization of the chlorophyll over the general surface of the leaf were unable to traverse the layer of suberized cells.

*L. c., p. 540.

†Aderhold (Bot. Centralbl., Bd. 89, 1902, pp. 204-206) has also recently corroborated the main points of Duggar's observations and collected some new data.

‡L. c.

All the phenomena observed by Blackman and Matthaei are readily explained on Duggar's hypothesis. A clean cut surface in a saturated atmosphere provokes no reaction in the leaf because the conditions for the growth of putrefactive bacteria, etc., are not then supplied. If, however, water is introduced, as by injection, and a foothold is obtained by bacteria or fungi, together with the possible injurious action of substances from the disrupted cells of the leaf near its injured surface, the reaction is readily called forth, and *is the result of the action of poisonous or deleterious substances.* Moreover, the reaction which these authors record as resulting from application of hot iron to a limited area is especially interesting. It is a well known fact that the destructive distillation of vegetable tissues in general gives rise to numerous poisonous compounds, which would readily diffuse into the adjoining healthy tissues and cause the formation of the absciss-layer in question.

Another phase of this exfoliation process deserves some attention. Any local injury of the character here considered causes the formation of an absciss-layer within tissues still living and turgid. As soon as communication is sufficiently cut off from the normal transpiration current by the suberized layer of cells, the occluded tissue begins to dry up and to shrink away from the healthy tissue, thus producing a certain tension at right angles to the plane of the absciss-layer and causing its early rupture. Fig. 1, plate V, before referred to, shows successive action of this character in the peach leaf.

In numerous experiments by the writer this exfoliation has never occurred as the result of a clean cut or puncture, but abrasion of a limited surface of the leaf, resulting in the accumulation of injured tissue, rarely if ever failed to produce the phenomenon. The only unmistakable cases of insect injury which has called it forth were caused by the larvae of the so-called slug, which in addition to leaving traces of its slimy secretion also leaves the dead veins and lower epidermis. The character of injury produced by different fungi on the peach leaf and its relation to this exfoliation process is quite interesting. Duggar found the effect produced on various leaves of the genus Prunus by a number of different fungi, including Cercospora, Cylindrosporium, Septoria, etc., and calls attention to the fact that others, as Exoascus, do not call forth the reaction. The writer has frequently examined leaves of the peach attacked by Exoascus and has never observed a case of exfoliation until after the death of the region attacked by the fungus, which furnishes additional proof of the correctness of Duggar's explanation for this phenomenon.

If the conclusion reached by Duggar is correct, it has a very important bearing on the question as to whether the copper in Bordeaux mixture, etc., enters the leaf in the soluble form through the epidermis. There can be no doubt that the various other poisons mentioned, including the secretions or excretions of fungi, are in direct contact with the assimilative cells on the inside of the leaf, and if copper in the form here under consideration provokes a similar reaction on the part of the leaf, it adds additional evidence of its absorption by the leaf.

The movement of starch toward this zone of abscission is illustrated in

Fig. 1, plate VII. There is in this healing process demand for materials out of which to construct the rapidly forming cell walls, and in accordance with well known physiological principles the starch is translocated to the region where it is demanded for formation of new cell walls, etc. A mere reference to Fig. 1, plate V, showing the actual exfoliation of an area surrounding a spot killed by copper, in connection with Fig. 1, plate VII, is sufficient to show the points here mentioned.

The writer has also made two experiments to determine whether the peach is specifically sensitive to the different factors producing leaf-fall.

On August 21, 1900, sets of leaves on five specimens each of apple, grape, and peach were selected, and all of the lamina of each leaf pinched away except a small area attached to the petiole, approximately the same amount of the blade being left in each case. Two days later the stubs of peach leaves in many cases snapped off at a mere touch, and within a week all were off or had loosely adhered after drying up. The stubs of the apple and grape leaves, on the other hand, not only remained attached about as long as uninjured leaves on the same plant, but the small remaining portion of the lamina maintained a healthy green color until the last observation was made about the end of September. If the simple mechanical removal of the greater part of the blade of a peach leaf will provoke in so short a time the formation of an absciss-layer as in the normal autumnal leaf-fall, it is not surprising that the removal of a large portion of the lamina by the action of a deadly poison should bring about the same result.

Reference has already been made to the rapid falling of peach leaves in the dark. Molisch* found the absence of light among the various factors producing defoliation. An experiment was made by the writer designed to determine whether the peach exhibits this reaction more readily than the grape or the apple, and at the same time to decide whether the presence of copper hydroxide on the leaves would hasten their fall. On August 22, 1901, a set of two seedlings each of apple, peach, and grape growing in pots were selected and photographed (Fig. 2, plate VI, upper row). Alternate plants were sprayed with the before mentioned copper hydrate mixture, and all were placed in a dark-room, where they remained until close of experiment. Four days later the peach leaves began to drop, one grape leaf also falling at this time. On August 29, after one week in darkness, the plants were taken out and again photographed. Their condition on this date is shown in lower row of pots, Fig. 2, plate VI. A glance at this cut shows that the peaches, sprayed and unsprayed alike, lost all their leaves except the youngest at the growing apex of the shoot. The apples, on the other hand, had lost no leaves by abscission. The sprayed grape plant lost all of its leaves, the unsprayed one had dropped two foliage leaves and the two tendrils it had formed. This difference in behavior of sprayed and unsprayed grape plants was evidently due to individual differences in sensitiveness. The experiment shows in a most emphatic manner that the peach responds much more quickly to the effect of darkness on leaf-fall than does the apple. As compared with the grape, the

*Sitzungsb. Akad. Wiss. Wien, Bd. 93, Abth. 1, 1886, p. 148.

peach also appears to lose its leaves under these conditions somewhat more quickly.

From these various considerations the following conclusions may be drawn:

1 The wound-healing in the peach leaf resulting in the formation of an absciss-layer and the exfoliation of a certain portion of living tissue, is the manifestation of a reaction on the part of the leaf toward various poisons coming into contact with the living tissues concerned.

2 This reaction is called forth in a striking manner by the action of copper spraying mixtures.

3 The method of wound-healing in the peach leaf does not differ essentially from that of the apple and grape leaf, if no infection results from organic or mineral poisons.

4 The removal of the greater part of the peach leaf mechanically or by exfoliation from action of poisons causes the rapid formation of an absciss-layer at the normal position for autumnal fall, while the apple and the grape leaf under similar conditions continue to assimilate with the limited remnant of the blade.

5 The peach leaf falls from the stem when exposed to darkness in far less time than does the apple leaf, and in somewhat less time than the grape; hence, the peach leaf must respond more readily to all the agencies resulting in formation of an absciss-layer than either the apple or the grape leaf.

6 This extreme sensitiveness of the peach leaf to various poisons, including copper, and to other stimuli causing leaf-fall suggests that the protoplasm of the leaf may be more sensitive to poisons in general than that of other tissues of this plant.

CHAPTER V

Influence of Copper on the Assimilative Function of Leaves

Rumm* found the chlorophyll content of grape leaves increased by spraying with Bordeaux mixture, and referred the earlier ripening of the fruit on sprayed vines, as well as its greater sugar content, to the greater assimilative activity of the leaves on the sprayed plants.† Frank and Krüger‡ experimented with potatoes in plots, spraying with Bordeaux mixture and milk of lime. These authors likewise found an appreciable increase of both chlorophyll and starch in the leaves sprayed with Bordeaux. Slightly greater assimilation was also noted in the leaves sprayed with milk of lime, but was so small as compared with the beneficial action of the Bordeaux mixture that there could remain no doubt of the beneficial effect of the copper. These authors also found an increased production of tubers in the sprayed plants, which they attributed to the increased assimilative activity of the leaves. Galloway§ found about the same time that nursery stock sprayed with copper mixtures shows a more vigorous growth than unsprayed stock, independently of the presence of fungous diseases. Galloway and Woods‖ conducted pot experiments with potatoes in the greenhouse, and concluded, as indicated by the harvest of tubers, that account must probably be taken of the fertilizing action of the lime by-products of the Bordeaux mixture which reach the soil. In these experiments it was unfortunately sought to determine the action of the copper constituent of the Bordeaux mixture by a check application of dilute copper sulphate solution, instead of the copper hydrate of the Bordeaux. These authors also admit the possible rôle of rain and dew in the action here under consideration.

The direct beneficial action of Bordeaux mixture on the host plant, especially in some cases, seems to be so thoroughly established that it has been generally accepted. The question remaining for solution is the immediate cause of this action and its mode of operation.

Aderhold¶ called attention to the fact that none of his predecessors had applied copper in a pure state to test its effects on the assimilative function of leaves. But Aderhold fell into the same error by using ammonium copper sulphate ("Azurin") for a "pure" form of copper, instead of the ordinary copper hydroxide occurring in the Bordeaux mixture. In the first place, the copper compound used in Aderhold's experiments is at

*Ber. Deutsch. bot. Ges., Bd. 11, 1893, pp. 79-93.

†Rumm (Fünfstücks Beiträge, Bd. 1, 1897, p. 83) proposed the term "sthenic" (*sthenischer Reiz*) for the stimulus here under consideration.

‡Ber. Deutsch. bot. Ges., Bd. 12, 1894, pp. 8–11; Arb. Deutsch. Landw.-Ges., Heft II, 1894.

§Bul. 7, Div. Veg. Path., U. S. Dept. of Agric., 1894.

‖Proc. Am. Assoc. Adv. Science, 1895, pp. 42–53.

¶Centralbl. Bakteriol., Bd. 5, 1899, pp. 217–220, 254–271.

first easily soluble, and if, after drying up on the leaf, this solution deposits copper hydroxide, another salt is present, ammonium sulphate, which must be taken into consideration. Aderhold made an investigation by spraying dwarf beans with Bordeaux mixture containing different proportions of iron, and found the starch content of the leaves increased roughly in proportion to the increase of iron content in the mixture, and concluded that the beneficial effects here under consideration are probably due to the iron contained as an impurity in commercial copper sulphate and lime. Aderhold tested starch in leaves by the well known method of Sachs,* viz., by dissolving out the chlorophyll and staining with iodine. He determined the relative starch content in different leaves by the relative intensity of the blue coloration with iodine solution.

When one considers the long known extremely poisonous action of copper on plants, and remembers that with the advent of an exceedingly small dose of copper in any form into a chlorophyll bearing cell all its vital functions, including that of assimilation, must suffer with an increased addition of copper, it is a little singular that no one has (so far as known to the writer) tested the action of chemically pure basic copper in this regard. This becomes only the more singular when it is remembered that the copper in properly made Bordeaux mixture occurs as the hydroxide. In the chemically pure hydroxide the copper is not combined with any radical (i. e., relatively speaking) which can seriously alter its toxicity or greatly affect its solubility. Moreover, the preparation of the substance (see p. 29) is not such a difficult matter as to deter one not expert in chemical manipulation from its preparation.

In the course of the investigation of the local injurious action of copper on the peach leaf (p. 31), it occurred to the writer that the same method there used might be applicable in the investigation of the effect of copper on the elaboration of starch by the leaf. It has already been shown that these spots formed by the deposit of copper hydroxide, especially when no lime accompanies it, endure unbroken for several weeks, though exposed to the disintegrating action of hard rains. It was hoped in this manner also to learn something more definite as to the exact conditions controlling the injury of copper to leaves; for not only must the assimilative function be impaired long before any evidence of death appears in the leaf tissues, but also any local reduction of assimilative activity must come out in sharp contrast on account of the extremely delicate reaction of starch toward iodine. That this action of copper sprayed on leaves, whatever be its character, must at least begin locally, follows of necessity from the insoluble or difficulty soluble character of the mixture applied and from the method of applying it. True, as has already been shown (p. 33), these spots slowly undergo disintegration or solution; but since they remain for so long a time visible to the eye, it is evident that their action, of whatever character, must begin locally.† Although the writer's investigations of this subject are not finished, and this discussion is to be regarded rather in the light of a preliminary announcement, the facts given below will show that

*Cf. Darwin and Acton, Physiology of Plants, 1894, p. 17.

†A similar local action of iron in restoring a healthy green color to chlorotic leaves was shown by Dufour (Zeits. Pflanzenkr., Bd. 1, 1891, pp. 136, 137).

some very important conclusions have been reached by the method here outlined. In the first place, attention may be called to the fact that any difference in starch content in a definite known region of the same leaf must appear far more distinctly to the eye than the mere cómparison of different leaves from different plants when held side by side, as was done by Aderhold* in his investigations. Moreover, as will be shown below, the individual element in different leaves, different exposure to light, and other characteristics not understood make any conclusions based on other than a large number of experiments unreliable, while, if under only a few spots of copper hydrate on the same leaf a distinctly greater or less intensity of coloration results than over the rest of the leaf, there can be little doubt as to the action of copper on this limited area. And whatever the effect of the copper on this limited area may be, must be true also of the entire leaf if covered wholly or partially with the same substance. Moreover, as was found by actual experience, more decisive results could be obtained by examination of the leaf surface in good light with the eye or with a lens, than by examination of a section through the region in question. Frank and Krüger† found that they could better estimate the relative chlorophyll content of leaves by comparing the intensity of color in an alcoholic extract, than by comparing under the microscope the chlorophyll bodies contained in individual cells, as had been done by Rumm.‡ In like manner, any slight difference in starch content must come out with greater contrast on inspection of a thick stratum of assimilative cells, as appears on superficial examination of a limited region of a leaf surface, than can appear on examination of a single layer of cells in section.

The question, however, occurs at once as to whether a spot of copper on a limited area of a leaf surface may not in some manner induce translocation of starch to or from the underlying region, and thus cause a greater or smaller amount of starch in this region at any given time. To this objection two replies may be offered, in so far as it relates to cases where much more starch is found directly beneath the copper spot. In the first place, there is satisfactory proof in the literature above cited that the foliage on a plant sprayed with Bordeaux mixture contains more starch than on one not thus treated. If this be true of the leaves of different plants, it must be true of the leaves of the same plant, or of different areas of the same leaf, under like conditions. A more striking evidence is seen in the special case under consideration. Reference has already been made to the striking evidence of translocation of starch in the peach leaf for the purpose of repairing an injury. In Fig. 1, plate VII, is shown one such case out of a number observed. The area of leaf here shown had copper hydroxide applied to lower surface on Aug. 24. At the end of four days the leaf was removed and put into alcohol, later into iodine solution. The blackened areas, *a*, *b*, and *c*, show parts killed before gathering; around two of these, *a* and *b*, as shown by lighter shading, the fatal action of the copper had invaded the surrounding region somewhat after the first portion was killed. This was clearly shown in the alcoholic specimen by the

*L. c.

†L. c.

‡L. c.

somewhat lighter brown color. The distribution of starch over this region at the time when the leaf was put into alcohol is clearly shown by the dotted shading. Not only does the intenser blue coloration indicate the region where the zone of abscission was to occur, as indicated by the accumulation of starch to meet the demands for rapid cell division, but *also the region from which this rapid translocation of starch has occurred is clearly denoted by a fainter coloration.* The contrast between the areas of accumulation and those of removal was even greater than that shown in the figure. This, therefore, furnishes experimental proof that where there is any marked accumulation of starch resulting from translocation, there is a surrounding region showing the loss of the starch thus carried away. In a large number of cases, partly tabulated on p. 81, where there was very great difference in content of starch between the region directly beneath the copper area and the surrounding portion of the leaf, there was no evidence that this starch had been carried there as a result of the action of copper.

It has been suggested by Sorauer* that a sprayed leaf shows a greater amount of starch than one not sprayed because the copper present retards the solvent action of the enzymes concerned in the conversion of starch into sugar. The fact already brought out by numerous investigations, viz., that the sprayed plant not only shows a greater starch content in its leaves, but also stores up more starch or other carbohydrates in its tubers or fruit, is a strong argument against such an assumption. Moreover, the writer has accumulated evidence which at least goes to show that the action of these solvent enzymes is not inhibited or even seriously checked by the copper. The case last mentioned is an argument in this direction. Strong evidence was brought out on p. 70 to the effect that this process of wound healing here under consideration is the result of toxic stimulation. If this assumption be true, the location of this zone of abscission must be determined by a certain strength of the diffusing poison — in this case, of copper. As shown in the figure referred to, starch has evidently moved radially from both directions toward the zone of accumulation. This translocation demands the action of these enzymes in the presence of copper which caused the injury.†

Again, it will be shown below that copper-dicupric hydroxide spots on peach leaves cause, after a very short lapse of time, considerable diminution in starch content of the underlying leaf area. Fig. 2, plate VII, shows the distribution of starch in the neighborhood of such a spot. Application was here made of $Cu_2O_3(OH)_2$ on August 24, the leaf being put into alcohol on August 28. In this same figure at *a* and *b* are shown two small areas *covered directly with the copper, in which the tissues had just died,* as shown by the faint brown color in the alcoholic specimen. Here is distinctly shown the accumulation of starch for the purpose of healing the wound, as before described. This, then, shows clearly that starch may be not only dissolved by the vital agencies in the leaf in the presence of copper and carried away, but it may also be redissolved by the same agencies and carried back.

*Zeits. Pflanzenkr., Bd. 9, 1899, p. 320.

†Cf. Pfeffer, l. c., Bd. I, pp. 507, 508.

It is now necessary to make a few statements as to the method of conducting these experiments. It was found best to use a medicine dropper drawn out to a fine point, so as to allow of the formation of very small round drops. Of the three mixtures used, copper hydroxide, copper-dicupric hydroxide, and Bordeaux mixture were thus applied in regular order, in rows running across the median region of the leaf. These rows were separated by a space of about 15 mm. When the leaves were gathered for testing, a circle of punctures was made around each spot by means of a cylinder of needles bound around a rod, the copper carefully washed off, and the leaf at once put into alcohol, or boiling water. Most of the starch estimations were made after the close of the season, from alcoholic material.

The different optical properties of the three mixtures thus tested must be taken into consideration, as actual results stated below indicate. The thin stratum of copper-dicupric hydroxide deposited by a drop of water containing this substance presents a dead black surface to the sun's rays, which must necessarily shut out a great deal of light, absorb heat, and elevate the temperature of the subjacent tissues of the leaf. The copper hydroxide spot is almost perfectly transparent, though slightly modifying the light passing through it. A thin stratum of copper hydroxide on an ordinary leaf surface shows by reason of its greenish blue color very little contrast with the rest of the leaf. Whatever may be the effect of the deposit of $Cu(OH)_2$ on the illumination of the underlying tissues, this effect must be far less than that produced by a similar deposit of either copper-dicupric hydroxide or Bordeaux mixture. Spots of the latter contain, in addition to $Cu(OH)_2$, also a considerable quantity of $Ca(OH)_2$, or $CaCO_3$, and $CaSO_4$. The effect of all these white lime compounds is to shut out a certain proportion of the sun's rays by reflection, rather than by absorption, as is the case with spots of pure $Cu_3O_2(OH)_2$. The Bordeaux mixture, therefore, would probably have no tendency to elevate the temperature of the subjacent tissues of the leaf, but its shading effect must evidently be taken into consideration. It is a well known fact that if a limited area of a leaf is shaded during exposure to sunlight of the remaining portion, the shaded region shows a distinctly smaller starch content than the illuminated region, which is sufficient apology for a somewhat detailed consideration of the optical properties of these different copper deposits as formed by the method above described. Furthermore, it is a well known fact that temperature modifies the action of poisons. Pfeffer* states that 'the action of poisons on turgescent plants is accelerated by an increase in temperature,' and Clark† found that fungi can endure the greatest dose of poison at or near their optimum growth temperatures. From the considerations just mentioned, it is clear that deposits of $Cu(OH)_2$ on the leaf will least affect both temperature and illumination of the tissues beneath; $Cu_3O_2(OH)_2$ will considerably reduce the action of light, but will have a distinct tendency to elevate the temperature of the subjacent tissues;‡ while the Bordeaux mixture will reduce the

*L. c., Bd. 2, p. 336.

†Quoted in Bot. Gaz., vol. 33, 1902, p. 80.

‡No experiments were made to determine whether differences in solubility of these compounds may be partly responsible for the results obtained.

amount of illumination slightly, but will probably have but little effect on temperature.

The contrast in the action of these three mixtures on the starch content of the peach leaf is rather striking. A number of experiments were made at different times on the leaves of trees, of seedlings in greenhouse, and on similar seedlings outside. Invariably, after exposure of only one day, less starch was distinctly visible immediately under each copper spot. The area covered by $Cu_3O_2(OH)_2$ invariably showed far less starch than the surrounding region; under Bordeaux there was distinctly less starch, but not so much contrast as in the case of $Cu_3O_2(OH)_2$; while, for the first day or two, there was very little difference between the starch content under $Cu(OH)_2$ and that in the surrounding region. In the latter case, however, within three days there was always a very distinct decrease in the amount of starch formed. It was shown in a previous chapter that both the pure basic copper compounds produce serious injury within a very short time, and from this consideration it is clear that a time must be reached before the death of the underlying region when there is less starch production, although the initial action of the copper may tend toward greater assimilation. A large number of peach leaves were tested in this way with almost uniform results, hence it is not necessary to describe any in detail. There was in a great many leaves exposed to outdoor conditions *a distinct halo of greater starch formation just about the margin of the spots*, especially of $Cu(OH)_2$ and $Cu_3O_2(OH)_2$. This can be explained, as it appears to the writer, only by assuming that these compounds have a tendency to increase the assimilative activity of the peach leaf, but owing to the special sensitiveness of this leaf to varying light conditions, *the shading effect of the copper spots has a tendency to overcome their stimulant action on the assimilative function.* Where these spots thin out at the margin, the immediately underlying tissues have more nearly normal illumination, and at the same time undergo the stimulant action of the copper, which results in this immediate zone in the formation of more starch than in the surrounding region of the leaf. A very convincing evidence for this assumed special sensitiveness to shading on the part of the peach leaf is found in the rapid dropping of these leaves in the dark, to which attention was directed on p. 72. In other cases, where there was not a specially evident halo of greater starch production around the spots of $Cu(OH)_2$ and $Cu_3O_2(OH)_2$, there were frequently visible *minute darker punctations* of various sizes, resulting from the reaction with iodine. A typical case of this sort is shown in Fig. 2, plate VII. This cut represents one of a number of similar spots of $Cu_3O_2(OH)_2$ applied to peach leaves on August 24, 1901, the leaves being put into alcohol four days later. For the reasons above stated, these black specks cannot be considered as marking zones of accumulation of starch, for in that event they would in every instance be surrounded by a zone of lighter color. Attention has already been called to the fact that, especially when exposed to rains, these copper spots on the peach leaf are frequently surrounded after the lapse of about one week by numerous red specks, which eventually wither and drop out. It thus appears that with sufficient illumination the first effect of copper on the assimilative cells of the peach leaf,

if not added in too great quantity, is to stimulate them to greater activity. It will be shown below that the apple leaf, and especially the grape leaf, responds very readily to this stimulant action of copper, and that more starch is formed even directly under the black areas of $Cu_3O_2(OH)_2$, but the contrast is still greater under the more transparent $Cu(OH)_2$. It was shown on p. 30 that spots of $Cu(OH)_2$ and $Cu_3O_2(OH)_2$ on leaves of the apple and grape after several weeks produce local injury just as on the peach, except that this injury is much longer delayed. If, therefore, the action of copper in the one case is at first beneficial, then after longer action injurious, one would expect the same in the other case.

It was above stated that the areas of the peach leaf directly under $Cu(OH)_2$ and $Cu_3O_2(OH)_2$ contained in most cases less starch than the surrounding region. One experiment, however, proved an exception, and is especially instructive in this connection. Late in the afternoon of August 2, 1901, five leaves on a peach seedling growing in pot received application of drops of $Cu(OH)_2$, $Cu_3O_2(OH)_2$, and Bordeaux, and were placed at once in the dark room alongside a similar plant untreated. The next morning similar spots were made on five leaves of the latter plant, and at 10:30 a. m. both were placed in the open sunlight. One spotted leaf from each plant was taken at the end of 20 minutes, 45 minutes, 1½ hours, two hours, and three hours, and placed at once in boiling water, preparatory to testing for starch distribution. The leaves on which the copper had been freshly applied showed no difference in starch content under any of the spots. Where the copper had been in contact with the leaf surface over night, however, *there was distinctly more starch under each spot of* $Cu(OH)_2$, *even at the end of* 20 *minutes.* In one or two cases there was doubtfully a little more starch under the $Cu_3O_2(OH)_2$ spots, but no distinct difference under the Bordeaux. This experiment indicates not only that some time is required for this stimulant action of copper, but that it is the same in the peach leaf as in the other cases investigated, the difference being simply a matter of time. It thus appears that the conclusion of Sturgis* that copper sprays reduce the chlorophyll content of peach leaves must be modified. The formation of more starch is the result of a greater chlorophyll content, as appears from the investigations cited above, and the incipient action of copper results in the formation of more starch, hence as a necessary condition of this increased assimilation, a greater chlorophyll content must be present. The destruction of the chlorophyll which Sturgis observed is simply one of the later stages of the toxic action of the copper on the peach leaf, which results, as shown above, in a modified form on other leaves as well.

A number of experiments similar to the above were made on the leaves of the grape and the apple. The increased assimilative activity of the region immediately under the copper was very marked on grape leaves, and the results obtained with them are decidedly more trustworthy than was the case with the apple leaves, although very distinct and unmistakable results were obtained with the latter. Still, in many cases, even grape leaves failed to show any appreciable contrast on treating with iodine. There are many causes which may be assigned for this difference of behavior in in-

*L. c., p. 54.

dividual leaves. As a rule, if one copper spot on a leaf caused a decided increase of starch in the subjacent region, a large percentage of the others showed a similar contrast. In most cases of failure there was too long exposure to sunlight, and the treatment with iodine resulted in too intense coloration to admit of any comparative determinations. In the table given below all such were eliminated, and from a number of leaves only those were chosen for a comparative study which seemed to be especially favorable. Probably the duration of exposure to sunlight is the sole determining factor in the case. Whether a distinct "image" will result, probably depends on the same conditions of "exposure" that determine the amount of "contrast" in a photographic negative.

In the accompanying table are brought together the results of one experiment on grape and apple leaves on fruiting plants in orchard and vineyard. On August 27, spots were applied as above explained to five shoots each on four apple trees, and the same number of twigs on a num-

TABLE II The Influence of Copper on the Assimilative Activity of the Leaf

Kind of Leaf	Number of days after Application	$Cu(OH)_2$					$Cu_3O_2(OH)_2$					Bordeaux				
		Number of spots	Less starch	Less with halo	No contrast	More starch	Number of spots	Less starch	Less with halo	No contrast	More starch	Number of spots	Less starch	Less with halo	No contrast	More starch
Apple, young, 8 leaves	1	32			3	28	69	33	6	15	42	37	34			68 (sl)
Apple, old, 7 leaves	1	20			45	55	30	10	37	23	30	35			49	51 (sl)
Grape, young, 2 leaves	1	7		43	14	43	3		100			3			34	66
Grape, old, 6 leaves	2	56			28	72	60	2	31	5	54	58		5	62	33 (sl)
Apple, young, 3 leaves	3	10		30	30	10	7	30	10		50	10			50	50
Apple, old, 4 leaves	3	13			38	62 (sl)	15	40	33	27		15			13	87
Grape, old, 5 leaves	3	17			6	94	23			30	70	29			76	24
Apple, young, 2 leaves	6	5	80		20		6	17	50	33		5				100
Apple, old, 3 leaves	6	10	30		70		11	64	36			10			40	60
Grape, young, 3 leaves	6	9			78	22	10		20		80	6			66	34
Grape, old, 5 leaves	6	17			29	71	21		48	9	43	23			52	48

ber of grape vines in vineyard. Care was taken in each case to select leaves with similar exposure, of similar development, etc. Representative leaves were gathered from each twig and subjected to iodine test, at the end of one, two, three, and six days. It was designed here to show also any possible difference in behavior between young and old leaves, but the lateness of season makes this estimate in the case of the apple unreliable. The grape leaves near the distal ends of the shoots were, however, plainly immature. In the first column is stated the kind of leaves from which the estimate was made. In the second column is given the number of days that elapsed between the application of the mixtures and the removal and examination of the leaves. The results observed from the action of the three mixtures, $Cu(OH)_2$, $Cu_3O_2(OH)_2$, and Bordeaux, are grouped together, as shown. In each case is given the actual number of spots from which the percentage estimates in the four following columns were made.

It is needless to repeat that each leaf contained some spots of each of the three mixtures used, and hence the successive columns are in every respect comparable. For convenience of reference, the number of spots under each set showing respectively less starch, less starch with halo of more, no contrast, and more starch, is expressed in percentage of whole number of spots examined in each case. All these points are appropriately designated at the heads of the columns. Where the contrast was particularly striking, an exclamation point is placed after the number, and where this contrast was very slight the abbreviation "(sl.)" is appended in parenthesis.

Before drawing any general conclusions from the experiment whose results are here tabulated, several facts must be borne in mind. In the first place, none of the general conclusions stated below are based simply on this one experiment, but on a number of other similar ones in addition. This table, as above stated, is based simply on the percentage of local areas on the same leaves which show a perceptible increase or decrease in starch content as a result of the action of the copper, but only very roughly in a few cases indicates the relative intensity of this action. As above stated, grape leaves show a far more distinct increase of starch under the stimulant action of copper than either the apple or the peach. This is especially true of the leaves that have passed maturity, but is also evident in many cases on young grape leaves. So great is this increase of starch in some cases, that the intensely blue spots are distinctly visible to the eye entirely across a room after the leaf has been stained with iodine. It is evident that the intensity of the action of copper on starch production must be considered of at least as much importance as the percentage of spots which show a greater or less starch production.

Based on this latter fact, it was evident that young leaves in most instances responded to this stimulus in less time than the older ones; although this does not readily appear from the tabulated results. On the whole, however, the experimental results on young leaves are too meager to admit of any definite conclusions. An especially interesting phenomenon frequently observed here was the apparent *wide diffusion* of the copper stimulus on the young leaves. Attention was called (p. 31) to the same evidence of diffusion in the distribution of injured areas around the copper spots on young grape leaves.

One of the most important conclusions to be drawn from the results here partly tabulated, is that *the action of pure copper hydrate on starch production for the first few days after its application is far more intense than that of the Bordeaux mixture*. Attention is again called to the fact that these spots of different mixtures were on the same leaf and necessarily under absolutely identical conditions. Not only did the green and transparent copper hydroxide show greater influence on starch production than the Bordeaux, but also in many cases, even the black dicupric hydroxide showed the same result, in spite of its shading action. Thus in the table the older grape leaves showed at the end of two days under $Cu(OH)_2$ spots much more starch in 72 per cent of these spots; under $Cu_3O_2(OH)_2$, a quite similar contrast in 54 per cent; while under Bordeaux only 33 per cent showed any appreciable increase of starch, and this increase was very faint in comparison with that under pure copper. The

same rule holds in a general way to the end of the experiment. But if this experiment had been extended over a longer period, a time would have been reached in the case of the grape leaves, just as in the apple leaves, as shown below, when there would have been less starch in every case under spots of $Cu(OH)_2$ and $Cu_3O_2(OH)_2$, but more under the Bordeaux spots. It has already been shown (p. 30) that this stimulant action of copper not mixed with lime, so beneficial at first, eventually becomes injurious and results in the death of some of the underlying cells. On running down the column under $Cu(OH)_2$ headed "more starch," it is seen that every grape leaf from the end of the first to the end of the sixth day contained a considerable percentage of spots showing more starch. A great many of these spots showed no contrast, and in a single case (two young leaves after one day) a few spots showed less starch with halo of more, but in no case did a single spot out of 106 examined show less starch. An examination of the corresponding columns under $Cu_3O_2(OH)_2$ will show a very similar result, although here the shading effect of the black copper compound is clearly indicated. Here will be seen in one case a few spots with less starch, some spots on nearly every leaf showing less with halo, and many showing no contrast. A comparison of the percentage of $Cu_3O_2(OH)_2$ spots showing more starch with the corresponding figures under $Cu(OH)_2$ shows that with only one exception there is always a smaller percentage of spots with more starch in the former than in the latter. This is unquestionably due to the shading effect of $Cu_3O_2(OH)_2$, as may be shown by taking the sum of the percentages under "more starch" and "less with halo," and then making comparison. "Less with halo" in many cases actually meant an increase of starch formation, as was clearly evident to the eye. A comparison of the corresponding figures for Bordeaux in the case of grape leaves shows nothing very striking except the fact, as stated above, that the increase of starch is far less in intensity than where the pure basic copper is used, and that in no case from beginning to end of experiment was a single spot found with less starch. In only a few spots on old grape leaves after two days there was less starch with halo of more. There was, however, in nearly every case a considerable percentage of spots that showed no contrast.

The behavior of the apple leaves as shown in the table referred to presents some very instructive points. Attention has already been called to the fact that the reaction here under discussion is far less intense in the apple than in the grape leaf, as shown by the iodine tests. Yet, it was found to be sufficiently pronounced to admit of accurate results. Inspection of the table will show essentially the same difference of behavior on the part of the apple leaf toward the several copper mixtures as was above shown for the grape leaf. For example, at the end of one day the older apple leaves showed more starch under 55 per cent of the $Cu(OH)_2$ spots examined, and under only 30 per cent of the $Cu_3O_2(OH)_2$ spots. This difference holds to the end of the third day, and may without further question be assigned to the same cause as was shown above in the case

of the grape leaf. But the most important fact here brought out is the striking difference noted in leaves gathered on the first and third days after application of the mixtures, as compared with those gathered from the same shoots on the sixth day. Leaves both young and old showed decidedly more starch under a large percentage of the spots of $Cu(OH)_2$ both on the first and on the third day; but on the sixth day, *not only were no spots found with more starch, but a very large percentage* (80 *per cent and* 30 *per cent*) *showed decidedly less.* Essentially the same is shown for $Cu_3O_2(OH)_2$. Attention has already (p. 30) been called to the fact that the leaves on these same shoots, left until October 14, showed the areas covered by $Cu(OH)_2$ and $Cu_3O_2(OH)_2$ in nearly every instance dead and parched, while the rest of the leaf surface was still in a healthy condition. The facts just stated show clearly that the injurious action of the copper was already in its incipient stage before the close of the sixth day after application. The Bordeaux spots, however, showed still about the same proportional increase of starch on the sixth day as on the first. Whether the Bordeaux would after a greater lapse of time have the same effect does not appear from these experiments. It thus appears very clearly that the stimulant action of copper resulting in an increased assimilative activity of leaves is exerted on all the leaves here investigated.

The difference of behavior observed is a difference merely in degree and not in kind. When copper hydroxide or copper-dicupric hydroxide is applied to peach leaves, it at first stimulates the assimilative cells to greater activity and causes an increase in starch production. In a short time, not determined by these investigations, this action, at first beneficial, becomes injurious, and depending on certain conditions above discussed, finally results in the death of the tissues involved.*

If lime is present this period between beneficial action and death is very much lengthened, or the injury perhaps entirely prevented. Exactly the same thing occurs in the apple leaf, except that the period between beneficial action and death is considerably longer than in the peach. The same may likewise be said of the grape, except that here the period in question is still longer than is the case with the apple. In all cases lime proves beneficial not because it *hastens* the action of the copper, but because it *retards* this action, which would finally destroy the foliage if unimpeded.

The principles here clearly brought out offer a very ready explanation for the results obtained by Aderhold,† who endeavored to show that the iron existing in commercial copper sulphate and lime is responsible for the action here under consideration. If Aderhold's leaves had been tested for starch within two or three days after spraying instead of at the end of five weeks, as he did, the results would very likely have been different. It is even possible that the ferric hydroxide formed in his mixtures had exactly the opposite effect from that which he assigned to it. Iron may retard

*The results here stated are seen to be in striking accord with the general principles laid down by Pfeffer (l. c., Bd. II, p. 339) as to the relations between a stimulant dose and a lethal dose of poison.

†L. c.

the action of the copper just as lime has been shown to do, and thus in the end result in the formation of more starch, not by its own action, but by preventing the action of the copper from reaching the injurious stage.*

These experimental results show conclusively that lime has a tendency to retard the stimulant action of copper which results in the elaboration of more starch, just as it also retards the injurious action of copper on the foliage. This is almost proof in itself that this beneficial action is merely the first stage of the toxic action of copper. This probability becomes only the stronger when it is remembered that other vital functions of both animal and plant cells are accelerated by minute doses of various poisonous substances† which in larger doses would prove fatal. The question that naturally presents itself in this connection is, whether the same external circumstances, such as the presence of water and weather conditions in general, have the same increased influence on the stimulant action of spraying mixtures on leaves as they were shown to have on the injurious action of these mixtures on peach leaves. The writer made several preliminary experiments to determine this question, and while the results so far obtained do not justify any definite conclusions, it can be said that after a number of tests with these various mixtures on the leaves of peach, apple, grape, and potato plants growing in pots in the greenhouse, not a single case was found where the starch content was increased. On the other hand, it was usually either diminished or not affected at all by these mixtures. In a few cases peach leaves seemed to show a halo of greater starch production around the margins of the copper spots, but this was very indistinct and doubtfully present. It can only be definitely stated that on trees outside, exposed to rain and dew, copper hydroxide in many cases caused a marked increase in starch production over the area covered with it, in the case of grape and apple leaves, but that this never occurred on seedlings of these plants in the greenhouse. It is hoped to extend these experiments to some definite results along this line during the approaching season.

In this connection, attention may be called to some of Sturgis'‡ results on spraying peaches. This author found the fruit on certain trees sprayed with various fungicides considerably injured, and attributed this injury to the direct action of the copper on the fruit, since he observed no injury to the foliage. In the first place, there are at least two cases on record§ where peach fruit was distinctly benefited by spraying. From the evidence presented above it is quite clear that diminution in assimilative activity may

*The writer does not wish to be understood as believing that iron may not have to some extent the same stimulant action on the assimilative cells as copper. In this connection it may be stated that the writer tested his stock of copper hydrate, used in all these experiments, for iron. The mixture was dissolved in excess of hydrochloric acid, a current of H_2S passed through this until all the copper was precipitated, and the clear, colorless filtrate tested for iron. There was not sufficient ferrous iron to give any coloration with potassium ferricyanide, but on oxidation and addition of potassium sulphocyanide, a very faint pink coloration resulted, indicating the merest trace of iron. This, of course, cannot have played any part in the phenomenon here under consideration—at least, in the sense in which Aderhold regarded it.

†Cf. Pfeffer, op. cit., Bd. II, pp. 339, 340.

‡L. c., pp. 252-258.

§See Quaintance, Ga. Exp. Sta. Bul. 50, 1900, pp. 250, 251; Townsend and Gould, Md. Exp. Sta. Bul. 71, 1901, pp. 119 et seq.

result long before any distinct injury to the leaves is visible to the eye. At the time of rapid growth of the fruit there is very rapid translocation of assimilates to the fruit, and if the leaves suddenly cease to supply this demand, it is but natural that the fruit should fail to develop or should even shrivel up.

In addition to its effect on the chlorophyll production of the plant, copper has been shown to increase the transpiration of leaves* and the rate of growth in fungi.† The writer made no investigations in this direction, but observed one point accidentally in the course of his experiments, which may be of some interest. Attention has already been called (p. 40) to a test with Bordeaux mixture and copper hydrate on water cultures of the peach. This plant succeeds only with difficulty when grown by the usual methods with water cultures, in this respect showing quite unfavorable contrast with the apple, and especially with the grape, both of which plants succeed very well in culture solutions. In the experiment described on p. 40, where some plants were grown in normal solution and some in Schimper's solution poor in lime, all the peach plants that had been sprayed with copper hydrate or Bordeaux mixture, both inside and outside, showed a very decided contrast in vigor as compared with unsprayed plants. They not only had a healthier appearance, but also were plainly transpiring more rapidly than unsprayed plants, as was shown by the necessity of frequently supplying distilled water to their roots. Even the plants which had insufficient lime and appeared at the beginning quite inferior to corresponding plants in normal solutions, appeared at the end of about a month decidedly more vigorous than the unsprayed plants in normal solution. The sets of plants were easily at this time grouped in the following order as to growth and vigor: (1) the sprayed cultures in normal solution, outside; (2) sprayed cultures in solution poor in lime, outside; (3) sprayed cultures in normal solution, inside; (4) sprayed cultures in solution poor in lime, inside. Lastly came cultures in normal solution inside, which were plainly inferior to the poorest of the sprayed cultures. Especially significant is the fact of the evident vigor of the sprayed plants outside, in spite of numbers of shot-holes in their leaves caused by the copper. These facts indicate that copper may have some effect on the leaves even in the absence of water, and that the presence of water may influence the action of copper hydrate on the assimilative function.

From the evidence here presented, the following conclusions may be drawn:

1 Copper hydroxide, copper-dicupric hydroxide, and the Bordeaux mixture at first increase the starch content of leaves to which they are applied.

2 The intensity of this stimulant action is modified to some extent by the degree of translucency of the deposit of copper left on the leaf. For this reason, $Cu(OH)_2$ is more marked in its influence on the assimilative activity of the leaf than $Cu_3O_2(OH)_2$. Bordeaux has decidedly less intense influence on this assimilative function than either form of pure basic copper, and this difference is not due to its shading effect.

*Frank and Krüger, l. c.

†Ono, Journ. Col. Sci., Imp. Univ., Tokyo, vol. 13, 1900, pp. 141-186.

3 While pure basic copper has at first a beneficial action on the leaves, resulting in the formation of more starch, after a certain lapse of time, depending on the specific properties of the leaf and weather conditions, injurious results ensue, finally ending in death to the tissues involved.

4 Lime accompanying the basic copper, as in the Bordeaux mixture, while tending to retard this beneficial stimulant action, also tends to retard or to prevent the subsequent injurious action. This retarding influence of lime thus exerts a very important beneficial action in the Bordeaux mixture, in that it causes a slight increase in starch production and neutralizes the injurious effects of the copper, resulting sometimes in an appreciable increase of harvest.

5 So far as the external influences are concerned, there is between the stimulant action and the toxic action of copper on leaves a difference merely in degree. The same circumstances, such as the presence of lime, probably weather conditions, etc., which intensify the one, also increase the other.

6 Where the copper in spraying mixtures enters the cells of leaves at the same rate, the interval between the appearance of the beneficial and the injurious action varies inversely as the specific sensitiveness of the protoplasm to the toxic action of copper.

7 Of the three plants here tested, the grape shows by far the most benefit from copper sprays; then follow in order the apple, and finally the peach, which is probably very rarely, if ever, benefited by spraying under normal conditions.

CHAPTER VI

General Conclusions and Practical Application of Results

From all the evidence presented on the preceding pages, there can remain little doubt that copper is absorbed by the leaves of plants sprayed with Bordeaux mixture, etc. Since the investigation of this question by Rumm* and by Frank and Krüger,† both of which investigations failed to reveal the presence of copper as indicated by the spectroscopic and the electrolytic methods of identifying this metal, it has been believed by many pathologists that the copper hydrate does not reach the assimilative tissues of the leaf. This, however, from the standpoint of the physiologist, presents such an anomaly that he is loth to accept the conclusion as final, in spite of the high standing of the investigators who promulgated the doctrine.

Of course, the most conclusive and the only final evidence of the entrance of the copper into the tissues of the leaf is to demonstrate its presence there by some appropriate test. The writer has made no experiments in this direction, but one line of investigation is planned out for the approaching season.

One of the first conditions permitting the absorption of any substance by the leaf is that this substance be either a liquid or dissolved in a liquid. This is not necessarily true of that portion of the leaf surface which contains stomata, but as was shown above (p. 32) copper hydrate produces injury on the upper surface, where no stomata occur; hence, so far as this discussion is concerned, the statement just made is true. In fact, investigation tends to show‡ that even gases will not traverse a cuticularized membrane unless they are soluble in its constituent water. It has been shown in this paper in the case of peach foliage that the injury caused by copper is of the same character, no matter whether this copper is supplied in the form of a soluble salt or of an "insoluble" hydrate. The most striking evidence bearing on this point is that the leaves of trees showing a marked individual susceptibility to soluble copper salts on their leaves, show the same susceptibility to copper hydrate or to copper in any other form. It has been shown that deliquescent copper salts are far more injurious to the leaves than salts which readily deposit on spontaneous evaporation of their solvent water. It has been shown that exactly the same conditions that determine the rapid absorption of water and dissolved substances by leaves, also accelerate the injury to peach foliage by copper hydrate. It has been shown that this injury does not occur at all if water does not accompany the copper on the leaves. All these facts, and many more pre-

*L. c.

†L. c.

‡Pfeffer, l. c., Bd. I, pp. 164–170.

sented in the body of this paper, *demand* for a rational explanation the solubility of the copper hydrate in the water standing on the leaf. The first question, therefore, to decide is, whether this copper hydrate is soluble in water. Frank and Krüger* found that the filtrate from Bordeaux mixture several weeks old produces no injury to the spores of various fungi. Rumm† found filtrate from Bordeaux to produce on filaments of Spirogyra no injury other than what might be expected of caustic lime. His experiments, however, as well as those of Frank and Krüger, on the question of the occurrence of soluble copper in the Bordeaux mixture, are not conclusive. Moreover, Rumm‡ had formerly found soluble copper in the ordinary Bordeaux mixture. Millardet found no soluble copper in the Bordeaux mixture until after 18 days exposure. The writer has not consulted all the literature on this subject, but on general considerations there can be little doubt that copper hydroxide free of calcium hydroxide and calcium carbonate is slightly soluble in pure water, and certainly so in meteoric water, which contains traces of ammonia and nitric acid. Rumm§ states that copper hydrate exercises no injurious action on Spirogyra longata unless the filaments come into direct contact with its solid particles, which would imply that the hydrate is insoluble in water. But Rumm does not give very plainly the method by which he prepared his copper hydroxide, other than that he made it from copper sulphate and lime. The writer does not know of any satisfactory method by which all the lime may be removed from copper hydroxide made by this method. Washing with water would eventually remove all the hydrate of lime, but long before the completion of the process much carbonate of lime must be precipitated on exposure to the air, to say nothing of the presence of the carbonate as an original impurity in the unslaked lime. It appears, therefore, very probable that copper hydroxide prepared by the writer's method (see p. 29) will dissolve in distilled water in sufficient quantity to produce injury to plant cells. As will appear below, the writer obtained some results which seem to indicate this. Barth suggested‖ that the fungicidal action of the Bordeaux mixture fails if too much lime is added, by reason of the fact that the cell sap from the leaves (?) cannot render the copper soluble. The writer has not seen Barth's paper, or the criticisms of it by Wortmann and Droop,¶ who are said to have shown his explanation untenable. It is probable that Barth reasoned on the same grounds as those proposed by Rumm** to explain the retarding influence of lime on the toxic action of copper hydroxide on Spirogyra. Rumm supposed that lime acts simply to neutralize, by reason of its strong basicity, an acid excretion from the cell, and thus prevents the solvent action of this acid on the copper hydroxide. So far as known to the writer, all results go to show that leaves impart to water on their sur-

*L. c., pp. 32, 33.

†Fünfstück's Beiträge, Bd. 1, 1897, pp. 81–156.

‡Ber. Deutsch. bot. Ges., Bd 11, 1893, p. 447.

§L. c.

‖Bot. Centralbl, Bd. 61, 1895, pp. 268, 269.

¶Zeits. Pflanzenkr., Bd. 10, 1900, p. 165.

**Jahresh. Ver vaterl. Naturk. Württemburg, Jahrg. 54, 1898, p. 326.

faces an alkaline rather than an acid reaction. At any rate, Gaudichaud* and Sachs† found this to be true in a large number of cases, and De Saussure‡ found that an appreciable percentage of their content of fixed alkalies may be washed out of leaves with distilled water. These facts probably constitute the argument used by Droop and Wortmann to show the incorrectness of Barth's theory.

The writer has shown above (p. 37) that the same evidence brought forward by Rumm to support his theory to account for the retarding influence of lime on the toxic action of $Cu(OH)_2$ on Spirogyra holds true also of foliage leaves. Lime greatly retards the injurious action of copper hydrate on leaves, as well as on Spirogyra. Moreover, Clark§ has recently extended the same observation to a number of fungi. Clark also found that a number of other organic substances, such as beet decoction, exert a solvent action on copper hydroxide. This author also observed that peach leaves exert a solvent action on copper hydroxide in the presence of water. The writer‖ recently pointed out the inadequacy of Clark's experiments as proof that leaves in general can exercise this solvent action on copper hydrate, by calling attention to the special secretion (p. 53) on peach leaves which may have exerted this solvent action. The writer showed on p. 54 that this secretion, even pure and in relatively large quantities, neither dissolves copper hydroxide nor increases the injury of copper to the foliage; therefore this difficulty, at least, is removed.

Before the appearance of Clark's paper the writer had independently made an experiment to decide the same question, viz., whether the peach leaf is instrumental in dissolving the copper hydroxide deposited on it. Dehérain and Demoussy¶ suggested that the extremely small dose of copper required to produce death in plant cells renders them an exceedingly delicate test for copper where every other poisonous substance is eliminated, and the same applies to cases where the only question is to determine whether copper is present in solution. In the writer's experiments Spirogyra filaments were employed to determine whether copper had passed into solution. After a number of different trials the following method was hit upon as giving the best results:

Discs about 12 mm in diameter were cut out of filter paper, and their margins dipped in melted paraffine, leaving a small area in the center uncoated. These discs were then fastened by means of paraffine to the open ground ends of short glass tubes, likewise paraffined inside and out. The liquid to be tested was dropped inside the tube upon the carefully cleaned bibulous area of the paper bottom, through which it ran and remained below as a hanging drop. Into this drop were put a few vigorous filaments of Spirogyra, cut into appropriate lengths and manipulated with paraffined needles. After thus preparing a set of such cultures, one to each filter tube, they were placed in a suitable frame and put under a moist bell jar.

*Comptes rendus Acad. Paris, t. 27, 1848, p. 35.

†Bot. Zeit., Jahrg. 20, 1862, pp. 264, 265.

‡Recherches chimiques, 1804, p. 287

§Bot. Gaz., vol. 33, 1902, p. 43

‖Bot. Gaz., vol 33, 1902, pp 244, 245

¶Annales agron., t. 27, 1901, p 559.

Microscopic examination from time to time indicated which cells were dead, and thus the relative amount of copper in solution could be roughly estimated by the rapidity of disorganization in the Spirogyra cells. Check cultures in distilled water indicated how long the cells could thus survive in the absence of copper. Two such experiments are here described in order to show whether the peach leaf allows of the escape of any substance having a solvent action on copper hydroxide.

The first experiment will be described in only a general way. The so-called "hardened" (*gehärtete*) filter paper was here used as above described. Copper hydroxide was applied as a spray to the surface of leaves or of glass in the usual way. These sprayed plants and glass surfaces were placed alongside in the sunlight, and the water in which the copper hydrate was applied was allowed to evaporate. These surfaces were then kept wet almost continuously for several hours in the sunlight by a dew-like deposit of distilled water, the object being to supply the conditions in which greatest injury occurs (see p. 47). At approximately the same time a similar quantity of this water was allowed to flow off the surface into clean watch glasses. From these it was put into the above described filter tubes, after which they were ready to receive the Spirogyra filaments. In the first experiment there were compared seven cultures from water collected from peach leaves sprayed with $Cu(OH)_2$; five cultures from similar leaves sprayed with distilled water only; four cultures in water from paraffined glass plate sprayed with $Cu(OH)_2$; five cultures in filtrate direct from stock mixture of $Cu(OH)_2$ and distilled water, which had been standing several months; and three cultures in distilled water. The filaments of Spirogyra were placed in these several fluids at 4:30 p. m., October 15, 1901. On the next morning, after 17 hours in the moist chamber, every cell in water from peach leaf sprayed with $Cu(OH)_2$ was dead and stained brown. Of the five cultures in distilled water from peach leaf, 44 per cent of the cells (as estimated) were in a healthy condition. Of the four cultures in water from paraffine surface sprayed with $Cu(OH)_2$, 84 per cent were dead. Of the five cultures in filtrate direct from stock mixture of $Cu(OH)_2$, all were dead, but the cell contents were not stained so intensely brown as those in similar cultures from peach leaf sprayed with $Cu(OH)_2$. Of the three cultures in ordinary distilled water an average of 50 per cent were alive and healthy.

This experiment indicates clearly (1) that there is no substance escaping from the peach leaf into distilled water on its surface which makes this water to any appreciable extent more injurious to Spirogyra cells than ordinary distilled water; (2) that Spirogyra cells die in considerably less time in water that has been standing in the presence of $Cu(OH)_2$ on peach leaves than in similar water from $Cu(OH)_2$ on a paraffine surface; (3) that the filtrate from a long standing mixture of $Cu(OH)_2$ and water is more poisonous than such water that has been in contact with $Cu(OH)_2$ for a short time, but probably less poisonous than similar water lying for a short time on a peach leaf covered with $Cu(OH)_2$. This experiment was repeated under almost identical conditions on October 17, with the same general results, except that in this case the filtrate from the stock mixture of $Cu(OH)_2$ and water proved to be quite as poisonous as that from $Cu(OH)_2$ on peach

leaves. A third experiment was made in the same general way on October 24. For the sake of clearer demonstration these results are tabulated in full below. Here are compared under absolutely identical conditions, cultures of Spirogyra in the filtrate from $Cu(OH)_2$ and water standing on peach leaf, $Cu(OH)_2$ and water standing on paraffine, and $Cu(OH)_2$ and water standing on glass. The Spirogyra filaments were applied at 12:30 p. m. and the results noted at 3:45 p. m.

		Condition of cells in each culture after 3¼ hours.
Spirogyra cultures in filtered drops of water from peach leaves sprayed with $Cu(OH)_2$	1	All dead.
	2	50 % "
	3	90 % "
	4	95 % "
	5	99 % "
	6	75 % "
	7	90 % "
	8	95 % "
	9	All " Av. 88 %
Spirogyra cultures in filtered drops of water from paraffined glass plate sprayed with $Cu(OH)_2$	10	20 % dead.
	11	30 % "
	12	30 % "
	13	35 % "
	14	10 % "
	15	35 % "
	16	20 % "
	17	30 % "
	18	10 % " Av. 24%
Spirogyra cultures in filtered drops of water from clean glass plate sprayed with $Cu(OH)_2$	19	60 % dead.
	20	60 % "
	21	20 % "
	22	50 % "
	23	40 % "
	24	90 % "
	25	5 % "
	26	80 % " Av. 51%

The table shows disorganization with 3¼ hours of 88 per cent of the cells in filtrate from peach leaf, 24 per cent in filtrate from paraffine surface, and 51 per cent in filtrate from glass surface. These figures, however, fail to express adequately the degree of contrast between the toxic action of the filtrate from the leaf surface and that from the glass or paraffine surface. In the former case the dead cells were usually stained a deeper brown color and the filaments as a whole were far less turgid, even where some cells were still evidently alive.* This difference of turgidity was manifest even to the eye on lifting out the filaments with a needle.

It is needless to mention that the same species of Spirogyra was used in each set of experiments. In some of the preliminary experiments, not referred to above, filaments of two or more species were sometimes put together in one drop, and the specific susceptibility to the copper poison came out very uniformly in the different cultures.

In order to determine whether there might be an escape of some acid

*Devaux (Comptes rendus Acad. Sci. Paris, t. 132, 1901, pp. 717–719) found that adjacent cells of the same filament of Spirogyra show striking differences in the rate of absorption of copper.

secretion or excretion from the cells of the leaf, as suggested by Rumm* for Spirogyra, the author kept drops of blue litmus solution on peach leaves for several hours in the sunlight, but no acid reaction was evident. Unfortunately, no test was made for an alkaline reaction, but in view of the above cited authorities, it may well be assumed not only for the peach leaf under such conditions, but for leaves in general.

In view, therefore, of the results of Clark† and of the writer just stated, it appears that there is *something* escaping from the peach leaf which has a solvent action on copper hydroxide. From the evidence before us the nature of this substance is not clear. But for the absence of an acid secretion from leaves, the suggestion of Rumm for the similar phenomenon in the case of Spirogyra would be satisfactory. This would also explain all the phenomena above shown with regard to the action of the carbonates of the alkaline earths in preventing the injury of copper on leaves. From the evidence before us it seems probable that the action of lime in preventing the injury of copper hydrate to algae and fungi as shown by Rumm and Clark is to be explained rather on the ground of its stronger basicity, thus preventing the solution of the copper by a purely chemical action. The same doubtless holds true of the action of lime in preventing plant roots from taking up copper solutions from the soil, as shown by Haselhoff.‡ Here the carbonate of lime must first precipitate the copper from $CuSO_4$, etc., in the form of the insoluble copper carbonate. The acid secretions of root hairs would doubtless take up the copper carbonate thus formed but for the presence of calcium carbonate. As to the action of lime, etc., on the leaves in preventing the injury by copper hydrate, the suggestion of Clark that it prevents in a somewhat similar manner the solvent action of nitric acid, etc., on the copper may be partly correct. It is also possibly true that the lime acts in some way to prevent the formation of double salts with the salts of the alkalies shown by De Saussure§ to be leached out of leaves by the action of water. At any rate, as indicated by the fact shown above that the carbonates of barium, strontium, calcium, and magnesium all show the same effect, the action appears to be a chemical phenomenon and not a biological one. One thing appears from these considerations quite evident; viz. that the leaves of the peach at least are able to dissolve copper hydrate, and that lime, having a tendency to prevent this solvent action, retards or prevents the injury of the copper to the leaves.‖ Lime was also shown to retard or prevent the injurious action of copper hydroxide on grape and apple leaves. The action here must be the same as in the case of the peach leaf, and the general conclusion is reached that copper hydrate exists in slightly soluble form on the foliage of plants sprayed

*L. c.

†L. c., pp. 42, 43

‡Landw. Jahrb., Bd. 21, 1892, pp. 263–276

§L. c.

‖Ferry (Rév. mycol., t. 16, 1894, pp. 142, 143) attributed the retarding influence of an excess of lime in the Bordeaux mixture to the greater affinity of the hydrate for CO_2, supposing that the copper of $Cu(OH)_2$ is brought into solution by the CO_2 contained in the air and produced by the normal process of respiration. This cannot be correct, for the carbonate of lime has the same effect as the hydrate

with Bordeaux mixture, etc., and that the carbonates of the alkaline earths have a tendency to retard this solvent action. With this fact established, every other phenomenon brought out in the experiments described on the previous pages becomes at once clearly explicable.

The action of copper fungicides on foliage in general may be reduced to three elemental factors: (1) the specific susceptibility of the leaves to copper poisoning; (2) the amount of copper gaining access to the protoplasm of the living cells within a given time; and (3) the temperature to which the leaf is exposed. Light probably has no direct influence on the action of poisons. It has been shown that in the sunlight, other things being equal, the injury of copper to peach leaves is far greater than in the shade, and the question here naturally suggested is, whether this injury is due to the more rapid action of the same amount of copper in the cells of the leaves in the sunlight* or to the advent of a larger dose of copper under these conditions. Since no injury whatever appeared in leaves even after a long time when they were not exposed to direct sunlight, the latter alternative seems to be the correct one. The changes of temperature may therefore, so far as they affect the toxic action of equivalent doses of copper already accessible to the living protoplasm, be left out of consideration.

The application of these principles to the explanation of the experimental results contained in the body of this work is a comparatively simple task. In the first place, the specific sensitiveness of the apple to copper poisoning shows clearly that, if exposed to the same strength solution of copper, it must perish in far less time than the grape or even the peach. At least, this holds true of copper applied to the roots, and there seems to be no reason why the same should not be true of the leaves of the grape and apple. As to the peach, however, there is good reason, as shown on p. 73, to believe that we have to do with a special *organic* sensitiveness to copper on the part of the leaves. This special organic sensitiveness of peach leaves to copper poisoning probably in itself goes far toward affording an adequate explanation for the great injury of fungicides to peach foliage.

Therefore, a direct comparison in sensitiveness to copper of the grape leaves with apple leaves can be made with greater certainty on the evidence presented in the first chapter of this work, than of either of these leaves with the peach. We may safely assume, especially on the grounds of the influence of copper on the assimilative activity of grape and apple foliage, as shown on p. 81, and further discussed below, that the protoplasm of the apple leaf is far more sensitive to the toxic action of copper than is that of the grape leaf.

The second factor influencing the effect of copper on foliage mentioned above, namely, the rate of entrance of copper into the protoplasm of the living cells, appears to be, so far as these investigations show, the most important one from the practical point of view. It is likewise the factor most subject to variation in response to differing external conditions.

The first and most important feature to be considered here is the solubility of the copper compound applied to the leaf. The question as to how this solution is brought about, whether by the meteoric water itself or by

*Aderhold (Bot. Centralbl., Bd. 89, 1902, p. 205) suggested that the excessive heating of the tissues in the sunlight is one of the causes of this injury.

certain substances escaping from the cells of the leaf, cannot be very fully discussed here on account of meager data. It has already been shown that at least in the case of the peach leaf something escapes which renders the copper of the copper hydrate more readily soluble in water. Whether there are any external conditions of moisture, temperature, light, etc., which influence the escape of these substances into the water on the leaf, nothing definite is known.* It is quite clear, however, that of two copper compounds added in the undissolved state to water standing on leaves, other things being equal, the more soluble one will enter the leaf more rapidly than the less soluble one. This would be the more evident where both substances are difficultly soluble.

In this connection attention may be called to the difference in action between so-called "strong" and "weak" Bordeaux mixtures. One frequently sees in horticultural literature the mixture thus referred to, as if the copper were all in solution. The only difference between a one per cent and a two per cent Bordeaux mixture properly made, so far as their fungicidal action is concerned, is that *the latter contains twice as much solid copper hydrate as the former.* From this consideration alone it is clear that a somewhat larger percentage of the leaf surface will be in actual contact with copper particles when a certain amount of the stronger mixture is applied to a similar leaf surface, than when an equivalent amount of the weaker mixture is applied to the same surface. Moreover, it is plain that the larger surface of the copper hydrate exposed to the solvent action of the water standing on the leaf in the case of the strong Bordeaux, will produce a saturated solution of the copper in less time than would occur in a weaker mixture. This latter condition would come into play probably more during alternating showers and sunshine, but from actual experiment the writer has found very little difference between the effects of half and full strength Bordeaux mixtures, and for the reasons here stated it may be assumed that the difference in percentages of copper used in Bordeaux mixtures has, except within wide limits, very little to do with the action of the contained copper on foliage.

From the evidence already presented, there can be little doubt that the carbonates of the alkaline earths act in some way simply to retard the solvent action of the water, etc., on the copper; hence, the excess of lime in the Bordeaux mixture has a tendency to retard the rate of entrance of copper into the leaf by simply preventing the copper from passing into solution. Temperature may be expected to modify the solvent action of water on $Cu(OH)_2$, as it does in the case of every other substance. But in the case under consideration temperature cannot play an important part because there is so little variation in this respect during the night, when dew is present on the leaf. Yet, this may become of some import during alternating showers and sunshine.

The next most important factor controlling the rate of entrance of copper into the leaf is the presence of water. It has been shown quite clearly that no injurious effects follow spraying the extremely susceptible leaves of the peach, provided they are not exposed to rain or dew. While all the efforts to show an increase of starch production in the absence of

*Cf. Clark, l. c., p 44. See also Büsgen, Bot. Zeit., Bd. 51, 1883, p. 87.

dew and rain failed in the author's preliminary experiments, it may yet be true that there is sufficient moisture in the cuticle of the living leaf to allow of the diffusion of a minute quantity of copper into the interior. In any case, it is very clear that the injurious action of copper will not occur in the absence of water. Especially conclusive are the results with deliquescent copper salts on peach leaves. Here the salt absorbs moisture from the rapidly cooling air in the afternoon and becomes a saturated solution just at the most favorable time for its entrance into the leaf by imbibition.

The last factor to be considered as affecting the rate of entrance of the copper into the leaf is the permeability of the leaf cuticle to the copper in solution. In the first place, there can be no question that the form of the copper compound in solution controls in part the entrance of the copper into the leaf. It is a well known fact that there are many substances which traverse with difficulty, if at all, a given membrane which is easily permeable to other substances. While no direct experiments have been made in the particular case here under consideration, it is probable that the smaller degree of injury produced by copper acetate than by equivalent amounts of copper sulphate on peach leaves is to be explained in this way. The cuticle has been shown to be not only of different thickness and of different degrees of permeability in the leaves of different species, but also individuals of the same species show a marked tendency to vary in this respect with the different conditions controlling transpiration. Not only does the cuticle vary in thickness and permeability according to the conditions of the plant's habitat, but also a given leaf varies in this respect according to the lapse of time since water stood on its surface. For this reason leaves of plants growing in an arid region, other things being equal, must be influenced more slowly by copper sprays than similar plants in a humid region. But just how far the manifest tendency of the leaves in an arid region to take up water by imbibition would offset the greater permeability of leaves in a humid region is a mere matter of conjecture. At the same time, the smaller amount of precipitation in an arid region must play a very important part.

A discussion of the passage of substances in solution through the cuticle demands the consideration of two very distinct processes; viz., osmosis and imbibition. That the former may occur follows very clearly from such experiments as the one by Wilson* above referred to. A bit of copper nitrate placed on a leaf surface causes, if the leaf is turgid, the exosmose of water from the tissues of the leaf; but if the leaf be in a wilted or semi-wilted condition, the current would move in the opposite direction until turgidity were restored, and would doubtless carry some copper in with it. Of course, no experiments have been made to determine which of these processes is the more responsible for the entrance of copper into leaves, but from the evidence presented it seems very probable that, so far as concerns the injurious action of copper hydrate on the peach leaf, the copper entering by imbibition is responsible. This action occurs especially toward evening, when the first dew begins to fall, and is more marked still during showery weather, which in the case of Bordeaux mixture, probably contributes to the injury by washing out the lime.

*L. c

It thus appears beyond question that, even after the partial solution of the copper hydrate, there are numerous factors, specific peculiarities of structure, weather and climatic conditions, etc., which must control the rate of entrance of the copper into the leaf. It now becomes necessary to examine the effect of the copper thus entering the leaf on its functions, as learned by the experiments described in the previous chapters. In the first place, the first visible effect of an extremely minute amount of copper on a chlorophyll bearing cell is to produce a stimulus resulting in the formation of more chlorophyll, and hence of more starch. If the supply of copper is kept up constantly at the same rate, the assimilative function may continue to show increased activity for a time, this time depending on the rate of entrance of the copper and the specific sensitiveness of the cells concerned. If the rate of entrance of the copper is sufficiently slow and the leaf is not very sensitive to the copper poison, the leaf may show increased assimilative activity to the end of its natural life, or may even have its life thus prolonged beyond the normal. But if the copper enters more rapidly, and especially if the leaf is at the same time very sensitive to copper poisoning, death or serious injury must eventually ensue.*

Thus, the grape leaf, which was shown to have a thinner and more permeable cuticle than the apple leaf, absorbs the copper from $Cu(OH)_2$ or $Cu_3O_2(OH)_2$ and shows as a result increased assimilation, at least up to the sixth day after application, but after a longer time death or serious injury results. If, however, lime accompanies the copper, injury may not result at all; in fact, by reason of the retarded solution of the copper the grape may show beneficial results from spraying even at the close of the growing season. In the apple leaf the cuticle is thicker and far less permeable, but the pure basic copper produces increased assimilation in as short a time as in the grape leaf, *because the apple is far more sensitive to copper than is the grape, and consequently a much smaller dose is required to call forth this reaction.* At the same time, before the sixth day after application, the pure basic copper has already begun to cause injury to the apple leaf, not because copper is entering at a more rapid rate than into the grape leaf, but because a far smaller dose of copper proves fatal to the apple leaf. Here likewise, after the lapse of a certain time, the leaf tissues die. This injury may be retarded or prevented by the use of lime, just as in the grape leaf. Lime here likewise makes the contrast in the starch formation less marked, but is beneficial because it prevents the entrance of sufficient copper to produce fatal results.†

*Another factor which must play some part here is the well known power of the ... nism to accommodate itself to gradually increased doses of poison.

†When one considers the extremely minute dose of copper necessary to show ... understood why all chemical methods, such as those used by Rumm and by Frank and Krüger, fail to show the presence of copper in the leaves. It was shown on page 25 ... a $CuSO_4$ solution 0.0000075 normal is fatal to water cultures of this plant in one ... This is equivalent to one part of metallic copper in about 25,000,000 parts of ... Coupin (Comptes rendus Acad. Paris, t. 132, 1901, pp 645-647) found one part ... sulphate in 700,000,000 injurious to wheat seedlings, and concluded that the ... plants thus indicate doses of copper which "chemical analysis is powerless to ... This becomes even more reasonable when it is remembered that the amount ... sufficient to produce the stimulus in question must be relatively far less than the ... just given. The probabilities are that it is almost infinitesimal in amount.

As applied to the experiments with peach foliage, we find the same principles holding. On the same leaf the injury is greatest at the margin because the more permeable cuticle is found there covering the glandular surfaces. It is greatest along the veins (as also in the apple and grape leaf) because the cuticle is more permeable there. It occurs at definite points under and around spots of copper hydrate because the cuticle happens to be more permeable at these points. The injury is more rapid on young than on mature leaves, because the young ones have a thinner cuticle and probably also because the young leaves allow the escape of a larger percentage of solvent substances, and are themselves covered with a secretion having the same effect. Peach leaves show greater injury when copper is applied to their lower surfaces because more copper enters there, just as more water does under similar conditions. The peach leaf, for reasons above stated (Chapter IV), has probably an *organic* sensitiveness to copper far exceeding the sensitiveness of the other organs of the plant. Therefore, it is only with difficulty that the conditions are furnished which result in the beneficial stimulant action of copper. This beneficial action on the peach is therefore very transient when compared with that on the grape or even on the apple. Yet, this action is the same in kind as that observed in the other cases. Here, as in the former cases, lime retards the injurious action of the copper, and doubtless would also be found to retard the stimulant action of copper if the proper conditions were presented.

The direct object of these investigations, as stated in the introduction, was to determine why peach leaves are so much more susceptible to injury than the foliage of other plants that are commonly sprayed for the prevention of fungous diseases. The full solution of this question has, of course, not been attained; but much light has been thrown upon it, and a statement may be made here of the results gained.

Peach foliage is very susceptible to injury by fungicides because—

(1) *Peach leaves (not the whole plant) are especially sensitive to poisons in general and to copper in particular;*

(2) *They have the power, which may or may not be possessed by the leaves of other plants, of dissolving copper hydroxide;*

(3) *They have a cuticle which is thinner and more permeable than that of some other leaves;*

(4) *They have glandular surfaces terminating their marginal teeth, which are especially fitted by reason of their thin cuticular covering for the absorption of copper in solution;*

(5) *They are especially sensitive to the various agencies producing leaf fall by a normal absciss layer;*

(6) *They have the power, in common with other leaves of the genus Prunus, in a similar manner to exfoliate any injured region, this exfoliation involving much more than the really injured cells. If this process removes a large portion of the lamina, the whole leaf drops.*

It is now proper, in conclusion, to offer some practical suggestions which follow from the foregoing investigations. There are two possible means of the use of lime in connection with the practical spraying of crops which constitute the only conclusions of a directly practical character that occur to the author.

It has been clearly shown that abundant rains greatly increase the injury of fungicides to foliage, and that the difference between the beneficial action of copper on the assimilative function of the leaves, in the one case, and the injurious action resulting in death to the tissues in the other case, is determined simply by the amount of copper which reaches the cells in a unit of time. It was also shown that the chief benefit of lime is in retarding the solution of the copper, so that it may not reach the protoplasm in sufficient quantity to produce injury. This action of rain, to which further investigation will no doubt add other meteoric conditions, beyond question goes far toward explaining the variant results obtained by different investigators as to the direct action of the fungicide on the host plant. In the case of such plants as the potato, an application of lime during a very wet spell of weather, following an application of Bordeaux mixture, might very appreciably increase the harvest of tubers by retarding the injurious action of the copper. Of course, it is to be remembered that fungicides are used primarily to combat fungous diseases, and the same agency under ordinary conditions that retards the action of the fungicide on the host will also retard its action on the parasite. In fact, the investigations of Rumm, Clark, and others on this very point go to show that a great excess of lime will retard the fungicidal action of the Bordeaux mixture. In any such use of lime as that just mentioned it is very plain that a fungous disease, which is always worse during wet weather, may produce far more injury as a result of a protective supplementary spraying of lime, than that produced to the host plant by the fungicide. Yet, circumstances may arise where the *intelligent* use of lime as suggested will prove beneficial.

But where most may be hoped for in the use of this method is in the treatment of diseases of the peach, plum, etc., whose foliage is so extremely susceptible to injury. Attention has already been directed to the experiment illustrated in plate VIII. In this case, however, lime was used subsequently to the application of the Bordeaux mixture, and hence covered all the copper with a layer of carbonate of lime. This was done on the supposition that the lime is washed out by the action of rains. While this is no doubt partly correct, it is very probable that this subsequent application of lime also *insures the presence of lime in contact with a larger number of copper particles on the leaf*. Evidently the problem in the use of fungicides on such a plant as the peach is, as far as possible, to prevent entirely the action of the copper on the host plant, and at the same time to expose the copper so that the spores of fungi dropping on the leaves and fruit may come into contact with it. Evidently, therefore, the lime ought to be applied before the Bordeaux mixture instead of after it. A thin layer of carbonate of lime on the leaves, resulting from a thorough application of thin milk of lime about two days in advance of the spraying with Bordeaux mixture, will unquestionably be a better protection for leaves against the injurious action of the copper than the above described application of lime following the Bordeaux. The Bordeaux will then lie above the stratum of calcium carbonate and can act on any fungus spores coming into contact with it. The investigations of Rumm on Spirogyra and of Aderhold on Fusicladium seem to show that this method may prove successful. These authors found that the copper hydrate in the Bordeaux mixture never failed

to destroy the cells in contact with it, or at least to inhibit the germination of spores.

In the case of the peach, however, an additional difficulty presents itself in the hairy covering on the fruit, which will unquestionably prevent the deposition of uniform layers of either the lime or the Bordeaux. In fact, any speculation as to the probable successful application of the method here outlined to the conditions of the commercial orchard is useless. It is hoped to test the matter at this Station during the approaching season.

BIBLIOGRAPHY

ADERHOLD, R.—Ueber die Wirkungsweise der sogenannten Bordeauxbrühe (Kupferkalkbrühe). Centralbl. Bakteriol., Bd. 5, Abth. II, 1899, pp. 217-220, 254-271.

ADERHOLD, R.—Ueber die Sprüh- und Dürrfleckenkrankheiten (syn. Schusslöcherkrankheiten) des Steinobstes. Landw. Jahrb., 1901. (By Ref. in Bot. Centralbl., Bd. 89, 1902, pp. 204, 205.)

BAILEY, L. H.—Impressions of the peach industry in western New York. Cornell Exp. Sta. Bul. 74, 1894, pp. 379-381.

BAILEY, L. H.—Experiences in spraying plants. Cornell Exp. Sta. Bul. 18, 1890.

BAIN, S. M.—Some experiments with fungicides on peach foliage. Tenn. Exp. Sta., vol. 8, Bul. 3, 1895, pp. 35-40.

BAIN, S. M.—The action of fungicides. Bot. Gaz., vol. 33, 1902, pp. 244, 245.

BAIN, S. M.—The injury of fungicides to peach foliage. Science, N. S., vol. 14, 1901, pp. 221, 222.

BARTH, —— —Einige neue Beobachtungen über die Blattfallkrankheit der Reben. Ldw. Zeits. für Elsass-Lothringen, 1894, p. 265. (By ref. in Bot. Centralbl., Bd. 61, 1895, p. 268.)

BARY, A. de—Comparative anatomy of the vegetative organs of the phanerogams and ferns. Oxford, 1884.

BERLESE & SOSTEGNI—Recherches sur l'action des sels de cuivre sur la végétation de la vigne et sur le sol. Rév. internat. de viticulture et oenol. 1895. (By ref. in Bot. Centralbl., Bd. 63, p. 270.)

BLACKMAN & MATTHAEI—On the reaction of leaves to traumatic stimulation. Annals of Bot., vol. 15, 1901, p. 553.

BOEHM, J.—Ueber die Aufnahme von Wasser und Kalksalze durch die Blätter der Feuerbohne. Landw. Versuchsst., Bd. 22, 1877, pp. 51-59.

BOUSSINGAULT, J.—Étude sur les fonctions physiques des feuilles. Ann. de Chimie et de Physique, sér. V, vol. 13, 1878, pp. 289-394.

BURGERSTEIN, A.—Uebersicht der Untersuchungen uber die Wasseraufnahme der Pflanzen durch die Oberfläche der Blätter. Jahresb. Leopoldst. Comm.-Real- u. Obergymnasiums Wien, XXII, 1891, pp. 3-47.

BUSCALIONI, L.—Un nuovo reattivo per l'istologia vegetale. Reprint from Malpighia, vol. 12, 1898 (?), pp. 1-20.

BÜSGEN, M.—Ueber einige Eigenschaften der Keimlinge parasitischer Pilze. Bot. Zeit., Bd. 51, 1893, pp. 53-72.

CHESTER, F. D.—Can peach rot be controlled by spraying? Del. Exp. Sta. Bul. 19, 1892.

CHESTER, F. D.—Experiments in the treatment of peach rot. Del. Exp. Sta. Rep. 9, 1897, pp. 20-30.

CLARK, J. F.—On the toxic properties of some copper compounds with reference to Bordeaux mixture. Bot. Gaz., vol. 33, 1902, pp. 26-48.

COPELAND & KAHLENBERG—The influence of the presence of pure metals upon plants. Trans. Wis. Acad., vol. 12, 1899, pp. 454-474.

COUPIN, H.—Sur la toxicité des sels de cuivre à l'égard des végétaux supérieurs. Comptes rendus Acad. Paris, 1898, pp. 2, 400.

COUPIN, H.—Sur la sensibilité des végétaux supérieurs à des doses très faibles de substances toxiques. Comptes rendus Acad. Paris, t. 132, 1901, pp. 645-647.

DARWIN & ACTON—Physiology of Plants, Cambridge, 1894.

DEHÉRAIN et DEMOUSSY—Sur la germination dans l'eau distillée: influence nocive de traces de cuivre. Annales agron., t. 27, 1901, p. 553.

DETMER, W.—Das pflanzenphysiologische Praktikum, Jena, 1895.

DEVAUX, H.—De l'absorption des poisons métalliques très dilués par les cellules végétales. Comptes rendus Acad. Paris, t. 132, 1901, pp. 717-719.

DROOP, H.—Ueber rationelle Behandlung des Weinstocks mit Kupferkalk (Bordeauxbrühe). Winzerzeitung 98, No. 18. (By ref. in Zeits. Pflanzenkr., Bd. 10, 1900, p. 165.)

DUFOUR, J.—Notiz über eine neue Art der Anwendung von Eisenvitriol bei gelbsüchtigen Pflanzen. Zeits. Pflanzenkrankh., Bd. 1, 1891, pp. 136, 137.

DUGGAR, B. M.—The shot-hole effect on the foliage of the genus Prunus. Reprint from Proc. 19th Ann. Meeting Soc. Prom. Agric. Sci., 1898, pp. 1-7.

DUGGAR, B. M.—Peach leaf-curl and notes on the shot-hole effect of peaches and plums. Cornell Exp. Sta. Bul. 164, 1899, pp. 371-388.

EBERHARDT—Action de l'air sec et de l'air humide sur les végétaux. Comptes rendus Acad. Paris, t. 131, 1900, pp. 193-196.

EDER, C.—Untersuchungen uber die Ausscheidung von Wasserdampf bei den Pflanzen. Sitzungsb. Akad. Wien, Bd. 72, 1875, p. 258.

FAIRCHILD, D. G.—Bordeaux mixture as a fungicide. U. S. Dept. Agr., Div. Veg. Path., Bul. 6, 1894.

FERNALD, C. H.—Experiments with Paris green on apple trees. Mass. Hatch Sta. Bul. 19, 1892, pp. 118, 119.

FERRY, R.—La bouillie bordelaise: préparation, môde d'action, indications, applications. Rév. mycologique, 1894, t. 16.

FRANK, B.—Die Krankheiten der Pflanzen, 2 Aufl., 1895.

FRANK & KRÜGER—Ueber den Reiz, welchen die Behandlung mit Kupfer auf die Kartoffelpflanze hervorbringt. Ber. Deutsch. bot. Ges., 1894.

FRANK & KRÜGER Ueber den directen Einfluss der Kupfervitriol kalk brühe auf die Kartoffelpflanze. Arb. Deutsch. Landw.-Ges., Heft 2, 1894, pp. 1-46.

GALLOWAY, B. T.—The effect of spraying with fungicides on the growth of nursery stock. U. S. Dept. Agr., Div. Veg. Path., Bul. 7, 1894.

GALLOWAY, B. T.—An experiment in the treatment of peach rot. U. S. Dept. Agr., Div. Veg. Path., Bul. 3, 1892.

GALLOWAY & WOODS—Spraying with fungicides as a means of increasing the growth and productiveness of plants. Reprint from Proc. Am. Assoc. Adv. Sci., 1895, pp. 42-53.

GARMAN, H.—An experiment on plum rot. Ky. Exp. Sta. Bul. 47, 1893.

GAUDICHAUD—(Alkaline reaction of water standing on leaves). Comptes rendus Acad. Paris, t. 27, 1848, p. 35.

GILLETTE, C. P.—Experiments with arsenites. Ia. Exp. Sta. Bul. 10, 1890, pp. 401-420.

GIRARD, A.—Recherches sur l'adhérence aux feuilles des plantes et notamment aux feuilles de la pomme de terre, des composés cuivriques, destinés à combattre leurs maladies. Comptes rendus Acad. Paris, t. 114, 1892, pp. 234-236.

GOUILLON & GOUIRAND—Sur l'adhérence des bouillies cupriques utilisées pour combattre les maladies cryptogamiques de la vigne. Comptes rendus Acad. Paris, t. 127, 1898, pp. 423-424. (By ref. in Hollrungs Jahresbericht, etc.)

GUTZEIT, E.—Bekämpfung der Kartoffelkrankheit und Steigerung des Knollenertrags durch Anwendung von Kupferkalkbrühe. Fühlings landw. Zeit. 1899, pp. 4, 5. (By ref. in Zeits. Pflanzenkr., Bd. 11, 1901, p. 152.)

HABERLANDT, Fr.—Das Austrocknen abgeschnittener und benetzter, sowie abgeschnittener und nicht benetzter grüner Pflanzentheile. Wiss.—prakt. Unters. Gebiete Pflanzenbaues, Bd. 2, 1877, pp. 130-137.

HABERLANDT, G.—Anatomisch physiologische Untersuchungen über das tropische Laubblatt. II, 2. Ueber wassersecernirende und absorbirende Organe. Reprint from Sitzungsb. Akad. Wien, Bd. 104, p. 62.

HALSTED, B. D.—Notes on Monilia fructigena and spore germination. N. J. Exp. Sta., 12th Ann. Rep., 1891, p. 288.

HARRISON, F. C.—The effect of spraying Bordeaux mixture on foliage. 23d Ann. Rep. Ont. Agr. Coll. Exp. Farm, 1898, pp. 125-128.

HASELHOFF, E.—Versuche über den Ersatz des Kalkes durch Strontian bei der Pflanzenernährung. Landw. Jahrb., Bd. 22, pp. 851-861.

HASELHOFF, E.—Ueber die schadigende Wirkung von Kupfersulfat- und Kupfernitrathaltigem Wasser auf Boden und Pflanzen. Landw. Jahrb., Bd. 21, 1892, pp. 263-276.

HEALD, F. D.—On the toxic effect of dilute solutions of acids and salts upon plants. Bot. Gaz., vol. 22, 1896, pp. 126-153.

HOFMEISTER, W.—Die Lehre von der Pflanzenzelle, 1867.

HOLLRUNG, M.—Handbuch der chemischen Mittel gegen Pflanzenkrankheiten. Berlin, 1898.

HOLLRUNG, M.—Jahresbericht über die Neurungen und Leistungen auf dem Gebiete des Pflanzenschutzes. Berlin, 1898 and 1899.

KAHLENBERG & TRUE—On the toxic action of dissolved salts and their electrolytic dissociation. Bot. Gaz., vol. 22, 1896, pp. 81-124.

KERNER & OLIVER—The natural history of plants, vol. 1.

KOHL, F. G.—Die Transpiration der Pflanzen und ihre Einwirkung auf die Ausbildung pflanzlicher Gewebe. 1886.

KOHL, F. G.—Kalksalze und Kieselsäure in der Pflanze, 1889.

LODEMAN, E. G.—Bordeaux mixture and the potassium ferrocyanide test. Garden and Forest, vol. 7, 1894, pp. 456, 457.

LODEMAN, E. G.—Spraying apple orchards in a wet season. Cornell Exp. Sta. Bul. 48, 1892, pp. 361-393

LODEMAN, E. G.—The spraying of orchards. Cornell Exp. Sta. Bul. 60, 1893, pp. 263, 302.

LODEMAN, E. G.—The spraying of orchards. *Apples, quinces, plums.* Bul. Cornell Exp. Sta., No. 86, 1895, pp. 104,134.

LODEMAN, E. G.—The spraying of plants. New York, 1897.

LOEW, O.—Bemerkung über die Giftwirkung des destillirten Wassers. Landw. Jahrb., Bd. 20, 1891, p. 235.

LOEW, O.—The physiological rôle of mineral nutrients. U. S. Dept. Agr., Div. Veg. Phys. and Path., Bul. 18, 1899.

LOTHELIER, A.—Recherches sur les tiges et les feuilles des plantes à piquants. Rév. gén. de Bot., t. 5, 1893, pp. 480-483, 518-528.

MOLISCH, H.—Untersuchungen über Laubfall. Sitzungsb. Akad. Wien. Bd. 93, 1886, pp. 148-184.

MÜLLER, Fr.—Blattlöcherpilz oder Kupferkalkwirkung. Schäden der Kupferkalkspritzung an Obstbäumen. Prakt. Blätter Pflanzenschutz. Jahrg. II, Heft 9, 1899, pp. 65-68. (By ref. in Zeits. Pflanzenkr., Bd. 11, 1901, p. 245).

NOACK, F.—Der Einfluss des Klimas auf die Cuticularisirung und Verholzung der Nadeln einiger Coniferen. Pringsheims Jahrb., Bd. 18, 1887, pp. 519-529.

ONO, N.—The influence of chemical agents on the growth of algae and fungi. Journ. Coll. Sci. Imp. Univ. Tokyo, vol. 13, 1900, pp. 141-186.

OVERTON, E.—Ueber die allgemeinen osmotischen Eigenschaften der Zelle, ihre vermutlichen Ursachen und ihre Bedeutung für die Physiologie. Vierteljahrsch. Naturf. Ges. Zürich, Jahrg. 44, 1899, pp. 88-135.

OVERTON, E.—Ueber die osmotischen Eigenschaften der Zelle in ihrer Bedeutung für die Toxikologie und Pharmakologie. Zeits. physikal. Chem., Bd. 22, 1897, pp. 189-209.

PFEFFER, W.—Pflanzenphysiologie, Bd. 1, 1897; Bd. 2, Hälfte 1, 1901.

PFEFFER, W.—Osmotische Untersuchungen, Leipzig, 1877.

REINKE, J.—Beitrage zur Anatomie der an Laubblattern, besonders an den Zähnen derselben vorkommenden Secretionsorganen. Jahrb wiss. Bot., Bd. 10, 1876, p. 117.

RICHARDS, H. M.—Die Beeinflussung des Wachsthums einiger Pilze durch chemische Reize. Pringsheims Jahrb., Bd. 30, 1897, p. 665.

ROLFS, P. H.—Some unexpected results of spraying peaches. Proc. Ann. Meeting Soc. Prom. Agr. Sc., 1895, pp. 40, 41.

ROSCOE & SCHORLEMMER—Treatise on Chemistry.

RUMM, C.—Zur Kenntniss der Wirkung der Bordeauxbrühe und ihre Bestandtheile auf Spirogyra longata und die Uredosporen von Puccinia coronata. Beitr. zur wiss. Bot., Bd. 1, 1895, pp. 81-156.

RUMM, C.—Die Giftwirkung der gegen die Peronospora viticola verwendeten Kupfervitriol-kalk-mischung (Bordeauxbrühe) auf Spirogyra longata. Jahresh. Ver. vaterl. Naturk. Württemberg. Jahrg. 54, 1898, pp. 322-327.

RUMM, C.—Ueber die Wirkung der Kupferpräparate bei Bekämpfung der sogenannten Blattfallkrankheit der Weinrebe. Ber. Deutsch. Bot. Ges., Bd. 11, 1893, pp. 79-93.

RUMM, C.—Zur Frage nach der Wirkung der Kupferkalksalze bei Bekampfung des Peronospora viticola. Ibid., pp. 445-452.

SACHS, J. von—Physiology of plants, London, 1887.

SACHS, J. von—Ueber alkalische und saure Reaktionen der Säfte lebender Pflanzenzellen. Bot. Zeit., 1862, p. 257.

SAUSSURE, Th. de.—Recherches chimiques sur la végétation, Paris, 1804.

SCHIMPER, A. F. W.—Zur Frage der Assimilation der Mineralsalze durch die grüne Pflanze. Flora, Bd. 73, 1890, pp. 207-261.

SCHIMPER, A. F. W.—Ueber Kalkoxalatbildung in den Laubblättern. Bot. Zeit., 1888, pp. 81-89, 113-123, 145-153.

SORAUER, P.—Neuere Studien über die Bordeauxmischung. Zeits. Pflanzenkr., Bd. 9, 1899, pp. 317-320.

STARNES, H. N.—Some peach notes. Ga. Exp. Sta. Bul. 44, 1898, pp. 209-250.

STUBENRAUCH, A. v.—Important details of spraying. Ill. Exp. Sta Bul 68, 1902, pp. 157-187.

STURGIS, W. C.—Peach foliage and fungicides. Conn. Exp. Sta. Rep. 1900, pp. 219-254.

SWINGLE, W. T.—Bordeaux mixture. U. S. Dept. Agr., Div. Veg Phys. and Path., Bul. 9, 1896.

THIELE, R.—Eine ungünstige Wirkung der Bordeaux-Mischung. Zeits. Pflanzenkr., Bd. 9, 1899, pp. 235-236

TOWNSEND & GOULD—Notes on spraying peaches and plums in 1900 Md. Exp. Sta. Bul. 71, 1901, pp. 115-127.

TSCHIRCH, A.—Das Kupfer vom Standpunkte der gerichtlichen Chemie, Toxicologie, und Hygiene. Stuttgart, 1893.

TUBEUF & SMITH—Diseases of plants. New York, 1897.

VIALA, P.—Les maladies de la vigne. Paris, 1893.

WATSON, W.—Absorption of water by dead roots. Ann. Bot., vol. 8, 1894, pp. 119, 120.

WIESNER, J.—Studien über das Welken von Blüthen und Laubsprossen. Sitzungsb. Akad. Wien, Bd. 86, 1882, pp. 209-265.

WILLE, N.—Kritische Studien über die Anpassungen der Pflanzen an Regen und Thau. Cohns Beiträge, Bd. 4, 1887, pp. 285-321.

WILSON, W. P.—The cause of the excretion of water on the surfaces of nectaries. Unters. bot. Inst. Tübingen, Bd. 1, 1881, p. 1.

WISSELINGH, C. van—Over cuticularisatie en cutine. Verh. koninkl. Akad. Wetens. Amsterdam, Dl. III, No. 8, 1894.

WOODS, A. F.—The destruction of chlorophyll by oxidizing enzymes. Centralbl. Bakteriol., Bd. 5, 1899, pp. 745-754.

ZACHARIAS, E.—Ueber Secret-Behälter mit verkorkten Membranen. Bot. Zeit., 1879, pp. 644 et seq.

DESCRIPTION OF PLATES

Plate I Toxic action of copper sulphate solutions on water cultures of Peach, Apple and Grape, after 14 days.

Fig. 1, Peach plants: First group to reader's left, in 0.0001 normal; second group, in 0.000075 normal; third group, in 0.00005 normal; fourth group, in 0.00001 normal; fifth group, in H_2O dist.

Fig. 2, Apple plants: First group, in 0.00006 normal; second group, in 0.00003 normal; third group, in 0.00001 normal; fourth group, in 0.0000075 normal; fifth group, in H_2O dist.

Fig. 3, Grape plants: First group, in 0.0005 normal; second group, in 0.0001 normal; third group, in 0.00007 normal; fourth group, in 0.00004 normal; fifth group, in H_2O dist. (See pp. 22, 23)—From photographs by author.

Plate II Fig. 1, Effect of constituents of the Bordeaux mixture on Peach foliage after 17 days. First pair of shoots sprayed with $Cu(OH)_2$; second, with $Cu(OH)_2+Ca(OH)_2$; third, with $Cu(OH)_2+CaCO_3$; fourth, with $Cu(OH)_2+CaSO_4$; fifth, with Bordeaux mixture. (See p. 37).

Fig. 2, Effect, after 12 days, of carbonates of barium, strontium, calcium, and magnesium applied to Peach foliage with copper hydroxide (See p. 40).

Fig. 3, Influence of rain and dew on injurious action of Bordeaux mixture on Peach foliage. Plants "inside" protected, plants "outside" exposed to dew and rain for 27 days after spraying. (See p. 46)—From photographs by author.

Plate III Influence of distilled water on injurious action of $Cu(OH)_2$ on Peach leaves. Upper row of leaves from potted seedling sprayed with $Cu(OH)_2$ and kept wet for six days; lower row from similar sprayed seedling kept dry. (See p. 46)—From photograph by author.

Plate IV Fig. 1, Marginal gland of Peach leaf, showing at *a* accumulation of secretion. (See p. 53). Magnification about 50 diameters.—From camera sketch by author.

Fig. 2, Upper epidermis of Apple leaf in cross section, designed especially to show cuticularization of outer wall. (See pp. 57, 59). Magnification 533 diameters.—From photomicrograph by author.

Plate V Fig. 1, Shot-hole effect on Peach leaf produced by copper-dicupric hydroxide after 12 days. (See pp. 31, 34, 71, 72). Magnification 12 diameters.—From photomicrograph by author.

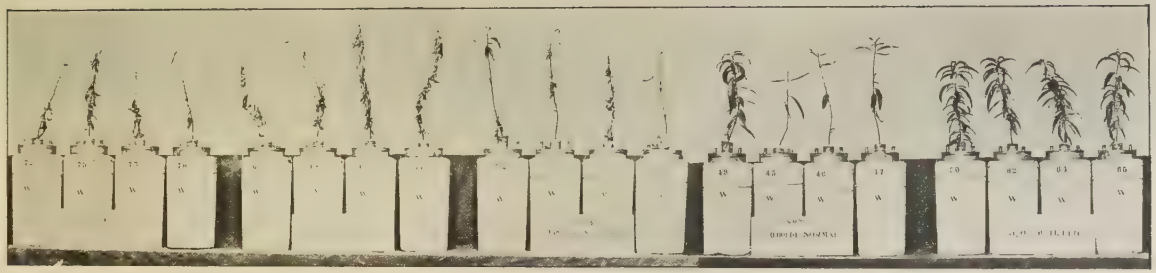
Fig. 2, Cross section of Peach leaf through margin, showing healing of artificial injury after 10 days. Region between *aa* and *bb* consists of cells with suberized walls. Magnification 286 diameters. (See pp. 69, 70).—From photomicrograph by author.

Plate VI Fig. 1, Marginal exfoliation of Peach leaf, caused by sulphur dioxide or sulphur vapor. (See p. 59).—From photograph by author. Fig. 2, Effect of darkness on leaf-fall of Peach, Apple, and Grape seedlings. (See p. 72).—From photograph by author.

Plate VII Fig. 1, Translocation of starch for healing injuries caused after four days by copper hydroxide applied to lower surface of Peach leaf. Distribution of starch indicated by intensity of shading. Dead areas indicated by black spots, *a*, *b*, and *c*. (See pp. 71, 72). Magnification about 7 diameters.—From camera sketch by author.

Fig. 2, Distribution of starch in vicinity of a spot of copper-dicupric hydroxide, after four days action on a Peach leaf. Copper area roughly indicated by unshaded space on interior, except about *a* and *b*, which are dead regions surrounded by starch rings. (See pp. 77, 79). Magnification 15 diameters.—From camera sketch by author.

Plate VIII Peach tree sprayed all over with half-strength Bordeaux. One-half of the crown, to the right, was given three subsequent applications of milk of lime, the other half left untreated. (See pp. 38, 47, 48).—From photograph by author.

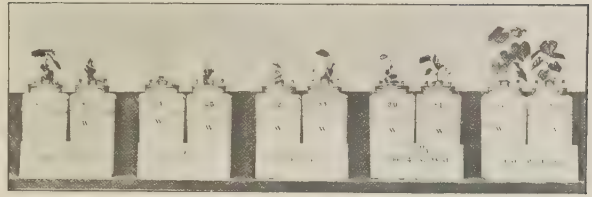


$CuSO_4$ 0.0001 0.000075 0.00005 0.00001 H_2O

FIG 1.

$CuSO_4$ 0.00006 0.00003 0.00001 0.0000075 H_2O

FIG 2.



0.0005 0.0001 0.00007 0.00004 H_2O

FIG 3

PLATE I.

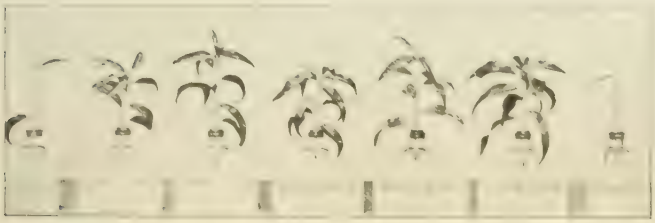


PLATE II

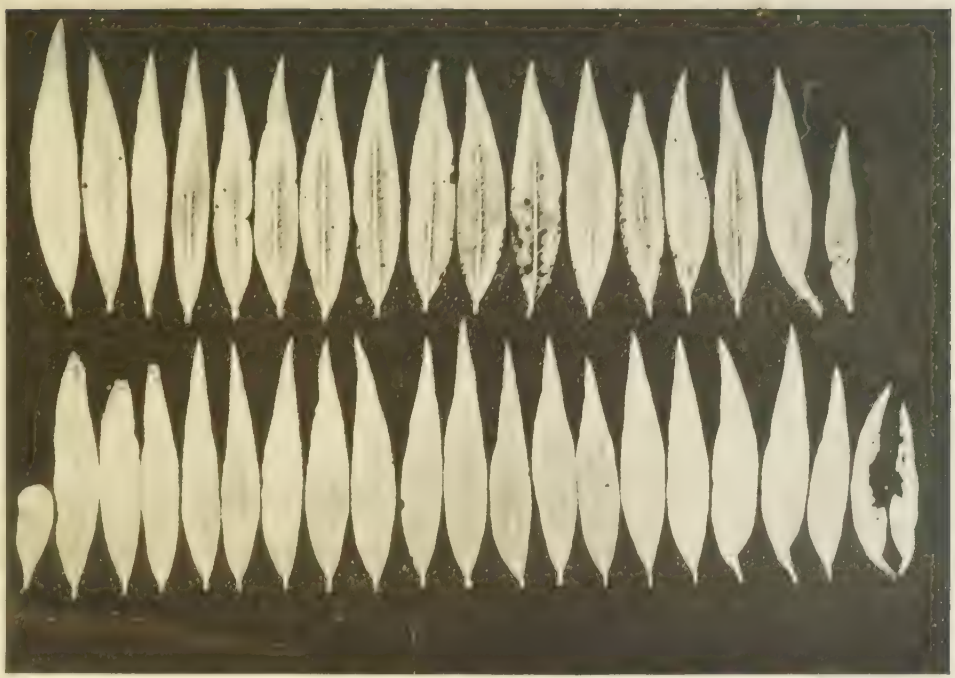


PLATE III

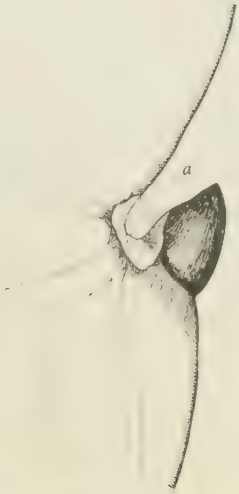


Fig. 1

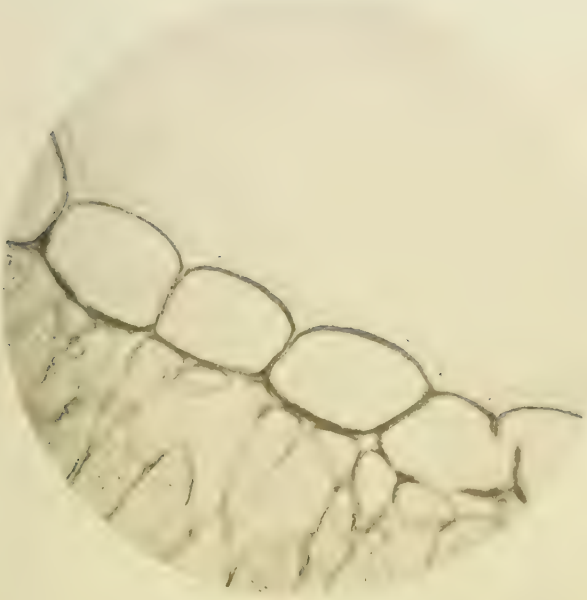


Fig 2.

PLATE IV.

Fig. 1.

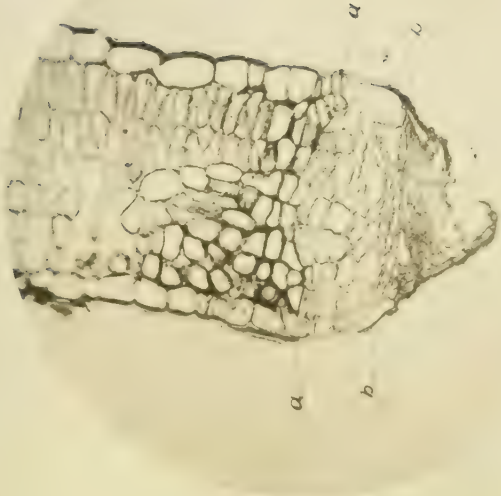


Fig 2.

PLATE V.

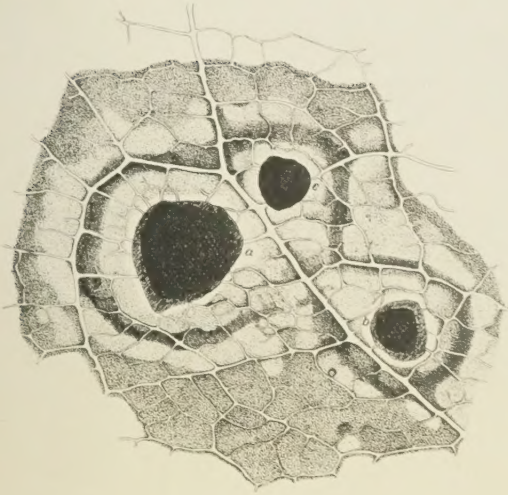


Fig 1.

Fig 2.

PLATE VII.

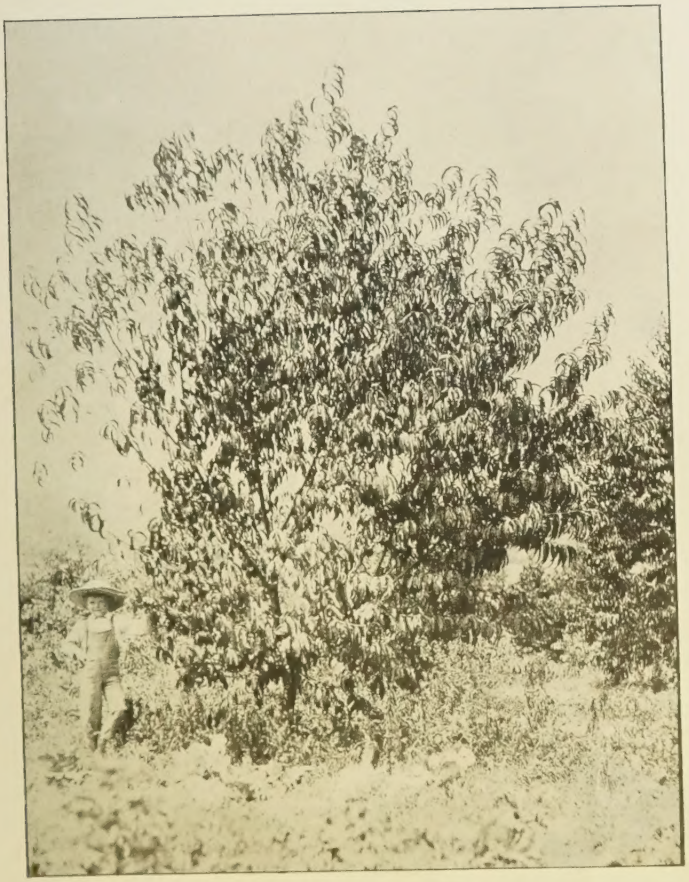


PLATE VIII.